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Office of
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Biotechnology

Minutes

Agricultural Biotechnology Research Advisory Committee

January 5 - 6, 1989

Document No. 89-01

U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL BIOTECHNOLOGY RESEARCH ADVISORY COMMITTEE
MINUTES OF MEETING

January 5-6, 1989

CALL TO ORDER AND APPROVAL OF AGENDA AND MINUTES

Dr. Bennie Osburn, Chair, convened the fourth meeting of the Agricultural Biotechnology Research Advisory Committee (ABRAC) to order on January 5, 1989 in Room 104-A of the U.S. Department of Agriculture (USDA) Administration Building, 14th and Independence Avenue S.W., Washington, D.C. The meeting was open to the public.

Members present included:

Bennie Osburn, Chair, University of California, Davis, CA;
Harold Hafs, Merck, Sharp and Dohme, Rahway, NJ;
John Gorham, Agricultural Research Service/Washington State University, Pullman, WA;
Ann Sorensen, American Farm Bureau Federation, Park Ridge, IL;
Fred Gould, North Carolina State Universtiy, Raleigh, NC;
Frank Whitmore, Ohio State University, Wooster, OH;
Nicholas Frey, Pioneer Hi-Bred International, Des Moines, IA;
John Kemp, New Mexico State University, Las Cruces, NM;
Sue Tolin, Virginia Polytechnic Institute and State University, Blacksburg VA;
Arnold Demain, Massachusetts Institute of Technology, Cambridge, MA;
Edward Korwek, Hogan and Hartson, Washington, DC;
Anne Hollander, The Conservation Foundation, Washington, DC;
Linda Phaire-Washington, Tuskegee University, Tuskegee, AL;
Alvin Young, Executive Secretary, USDA Office of Agricultural Biotechnology, Washington, DC.

Alternates in attendance included:
Anne Vidaver, University of Nebraska, Lincoln, NE;
Jeffrey Gibbs, Mackler, Cooper, and Gibbs, Washington, DC;
Ariel Hollinshead, George Washington University, Washington, DC

The roster of the Committee members present is included as Appendix A.

USDA Office of Agricultural Biotechnology (OAB) staff present included: Daniel Jones, Maryln Cordle, Marti Asner, Phillip O'Berry, Bert Wenner, Gary Weber, Eva Russnak, Barry Stone, and Elsie Brown.

Others present for all or part of the meeting included:

Orville Bentley, USDA Assistant Secretary, Science and Education;

Alex Thiermann, USDA Agricultural Research Service;
T. Stoner, DuPont de Nemours Company;
Frank Serdy, Monsanto Company;
Neville Clark, Texas A&M University;
L. Garry Adams, Texas A&M University;
Michael Olexa, USDA Extension Service;
Graham Purchase, Mississippi State University;
David MacKenzie, USDA National Biological Impact Assessment Program;
Paul Stern, University of Florida;
Kathleen Merrigan, Senate Agriculture Committee;
Greg Thies, Senate Agriculture Committee;
Mike Vandenbergh, Hogan and Hartson;
Allan Dietz, Senate Environment and Public Works Committee;
Keith Belton, American Chemical Society;
Susan Ely, ICI Seeds, U.K.;
Edward Debus, USDA Marketing and Inspection Services;
Terry Medley, Animal and Plant Health Inspection Service;
Althaea Langston, Animal and Plant Health Inspection Service;
George Shibley, Animal and Plant Health Inspection Service;
Charles Dangler, Pennsylvania State University;
Margaret Mellon, National Wildlife Federation;
Jane Rissler, National Wildlife Federation;
Janet Shoemaker, American Society for Microbiology;
Rudy Wodzinski, American Society for Microbiology;
Joel Cohen, Agency for International Development;
R.G. Brown, University of Massachusetts;
Rich Lotstein, Ciba-Geigy;
Manuel Barbeito, USDA Agricultural Research Service
T. Ross Wilkinson, Resident Instruction Committee on Organization and Policy;
C. Ward, Beveridge and Diamond;
Richard Parry, USDA Agricultural Research Service;
Rebecca Goldburg, Environmental Defense Fund;
J.S. Worthom, W.R. Grace and Company;
J.F. Quillen, French Embassy;
Jeffrey Fox, ASM News and Bio/Technology;
Jon Harsch, USDA AgriData News Service;
Gary Crawford, USDA Radio Division.

Dr. Osburn called the meeting to order at 9:10 a.m. He introduced Dr. Arnold Demain from MIT who was attending as an ABRAC member for the first time. Dr. Osburn also noted the presence of ABRAC alternates Dr. Ann Vidaver and Mr. Jeffrey Gibbs who were attending as resource persons. Dr. Ariel Hollinshead filled in as the designated alternate for Dr. Linda Phaire-Washington until her arrival which had been unavoidably delayed.

Dr. Osburn called the attention of the Committee to the preliminary agenda. The Committee approved the agenda as distributed.

Dr. Osburn introduced Dr. Orville Bentley, Assistant Secretary for Science and Education. Dr. Bentley welcomed the Committee to its fourth meeting and reiterated the importance of the ABRAC mission. He indicated that ABRAC is one of the most important advisory committees in Science and Education because of the public interest in biotechnology and the perceived risk of some biotechnology research activities. He encouraged the Committee to move forward with the research guidelines revising them as necessary, but he cautioned that the Department cannot hold up decision-making in biotechnology research until all the final answers are in. He concluded by saying that USDA looks to the Committee for the best scientific advice in the development of guidance for researchers in agricultural biotechnology.

Dr. Osburn called the attention of the Committee to the minutes of the previous meeting. Committee members enumerated several specific changes to the minutes. Dr. Demain called attention to several spelling errors in a list of scientific names in Appendix I of Appendix E and suggested that they be corrected. Dr. Puchase suggested that the minutes are a record of what actually transpired and that Appendix E should be retained as received by the Discussion Group on Classification of Organisms. Dr. Tolin attributed the misspellings to typographical errors made in transcribing the names from a photographic slide. Dr. Young indicated that the Office of Agricultural Biotechnology (OAB) staff would make an effort to correct the misspellings of the scientific names, perhaps retaining both versions for the record. The Committee voted unanimously to accept the minutes of the previous meeting with the corrections made by the Committee.

RESEARCH GUIDELINES

Dr. Osburn turned the Committee's attention to the draft research guidelines. He asked Dr. Tolin to present the results of the Guidelines Working Group meeting of December 2, 1988.

Dr. Tolin distributed a one-page Summary of Guideline Working Group Recommendations (Appendix B). She indicated that the classification of modified organisms for purposes of determining appropriate confinement levels in field tests continues to be problematic. She referred to one alternative, a matrix approach proposed by Dr. Sederoff at the December 2, 1988 Guidelines Working Group meeting (Appendix C). She indicated that in its current form, the matrix approach departs significantly from the categories in the guidelines. She reiterated three possible types of status changes an organism could undergo as a result of genetic modification; no change, increase in status, and decrease in status. Dr. Tolin concluded by saying that the Guidelines Working Group was open to suggestions from the ABRAC on this question.

Dr. Osburn invited Dr. Whitmore to comment on the draft guidelines. Dr. Whitmore had previously submitted written comments on the draft guidelines (Appendix D). He characterized the development of the status concept leading to the matrix approach as a breakthrough for the guidelines. He supported a reduction in the number of classes of organisms from 5 to 4 and recommended addition of a new type of genetic modification, namely, a genetic change resulting in organisms that are no longer able to express a normal trait considered hazardous to human health or to managed or natural ecosystems. Dr. Whitmore observed that the Texas A&M Brucella proposal exemplified such a genetic change. He concluded by noting that his table of classifications was similar to that of Dr. Sederoff.

Dr. Osburn invited Dr. Hafs to comment on the draft guidelines. Dr. Hafs commended the Guidelines Working Group for their efforts. He saw two remaining needs, however, to flesh out the confinement levels for different kinds of genetic changes and to incorporate the tabular approaches of Dr. Sederoff and Dr. Whitmore in a way agreeable to the Committee.

Dr. Osburn invited Mr. Stern to comment on the draft guidelines. Mr. Stern had previously submitted written comments on the guidelines (Appendix E). He referred to the need to define "harm" if certain kinds of terminology are used in the guidelines. Dr. Hafs questioned whether the guidelines could be designed realistically to prevent harm. Mr. Stern replied that the goal of the guidelines should be to reduce uncertainly rather than to prevent harm. Ms. Hollander gave weediness, toxin genes, and herbicide resistance in unintended hosts as examples of harmful outcomes. Dr. Frey supported new wording that would focus on promoting safe research rather than preventing harm. Dr. Tolin supported new language to replace the term "unreasonably adverse effects" in previous drafts. Dr. Korwek supported the changes proposed by Mr. Stern as salutary and improvments to the internal consistency of the guidelines.

Dr. Osburn invited Dr. Hollinshead to comment further on the guidelines. Dr. Hollinshead proposed a number of specific changes in the text of the guidelines. She commended Dr. Sederoff and Dr. Whitmore for their comments, but expressed support for 5 categories of organisms rather than 3. She reiterated that the handbook for field testing should describe the levels of confinement.

At the Chair's request, Mr. Stern introduced new text for procedures to amend the guidelines. Under these procedures anyone could request changes in the guidelines and the ABRAC would review the appropriateness of the proposed amendments.

Dr. Osburn invited other Committee members to comment on the draft guidelines. Dr. Korwek questioned the description of Status 1 in the classification of organisms. He also raised a

more general question about the impact on biosafety decision-making of classifying organisms. He drew a distinction between the characteristics of organisms and the effects of organisms. He said that the effects of a genetically modified organism may depend as much or more on the context or environment of a test as they do on the characteristics of the organism.

Dr. Tolin pointed out that Section IV-B of the guidelines addresses environmental considerations. She said the current assignment of the status of an organism is intended to be independent of the environment in which a organism might be tested. In her view, this was consistent with comparable language in the the National Institutes of Health (NIH) Guidelines and an Office of Technology Assessment (OTA) report on environmental release.

Dr. Kemp expressed support for Dr. Tolin's view. Ms. Hollander expressed support for Dr. Korwek's view. She said the current classification of organisms confuses their effects and characteristics, that the classification should be simpler, and that more examples should be given. Mr. Stern indicated that the language dealing with transfer, dissemination, and reproduction came through the Guidelines Working Group from NIH and OTA sources and that there was an important difference between classifying an organism and classifying an experiment.

Dr. MacKenzie expressed the view that a large matrix was confusing and he proposed an alternative (Appendix F). It consisted of three kinds of status and two 2 X 3 matrices for arriving at level of review required and probable confinement level. Ms. Hollander said that approach was interesting, but she questioned classification by risk according to genetic modification.

Dr. Purchase questioned Dr. Korwek's view on the effects/characteristics issue. Dr. Purchase cited foot and mouth disease as an example in which the etiologic agent spreads well, but does not kill very many cattle. He viewed pathogenicity and replication/survival as separate and not necessarily sequential processes. Dr. Korwek said he was concerned about status classifications that dealt with both parameters and conclusions. Dr. Purchase questioned that concern and suggested that the potential for causing harmful effects is not a conclusion. Ms. Hollander suggested that the Committee needed a compromise between the approaches of Dr. Purchase and Dr. Korwek in order to address the ability of organisms to cause harm in addition to their dissemination, reproduction, and other characteristics. Dr. Korwek acknowledged that he may not be in disagreement with Dr. Purchase, and that it would be easier to react to something in writing.

Dr. Osburn asked Drs. Tolin, MacKenzie, Purchase, Whitmore, and Mr. Stern to meet during the lunch break and try to resolve any differences they might have regarding the guidelines.

INTRODUCTION TO FIELD TESTING

Dr. Osburn turned the attention of the Committee to the Introduction to Field Testing (IFT), formerly "The Handbook." Dr. MacKenzie updated the Committee on the IFT. He said that various activities of the National Biological Impact Assessment Program (NBIAP) will complement the IFT. He envisioned three levels of publications; the IFT, a work on the "Principles of Conducting Field Testing," and several group-specific publications on organisms such as nightshades and pond-contained fish. He said he hoped that the National Technical Information Service (NTIS) would publish and distribute the "Principles" and group-specific publications. Dr. MacKenzie solicited volunteers to review drafts of the organism group-specific publications. Dr. Hafs, Dr. Gould, and Ms. Hollander volunteered to review specific drafts.

Dr. Young asked the Committee members if, in their view, the IFT should include chapters on public relations, socioeconomic impacts, and bioethics. Dr. Gould expressed the view that the Department rather than the ABRAC should address the public relations and socioeconomic aspects of biotechnology. Dr. Hollinshead questioned the need for a chapter on ethics in a biosafety manual. Dr. Vidaver expressed the view that public relations, socioeconomics, and ethics go well beyond the basic aspects of field testing. On the other hand, Dr. Kemp expressed reluctance to separate those chapters from the IFT if their prompt publication elsewhere could not be guaranteed.

Dr. Osburn suggested that OAB might submit the entire draft, including the chapters on public relations, socioeconomics, and bioethics, to the Committee for a final determination of their suitability for the IFT at the March, 1989 meeting. A Committee member so moved and the Committee approved the motion unanimously.

RESEARCH GUIDELINES

Dr. Osburn returned the Committee's attention to aspects of the research guidelines which had not been concluded earlier.

Dr. Tolin moved to accept the December 5, 1989 draft of the USDA Guidelines for Research Outside the Laboratory Involving Biotechnology (Document 72) with the following modifications:

Section I. Accept, with substitution of the first paragraph from page 2 of Document 73 for the first paragraph of Section I in Document 72.

Section II. Accept, with deletion of reference to Appendix A, and additional modifications to be finalized. These involve:

In II-A, accept Statuses 1-5, but change "and's" and

"or's" to clarify intent, with changes based on those proposed in Document 73, pages 5-7.

In II-B, accept Dr. Whitmore's suggestion of adding a fourth type of genetic modification as Type 1: "II-B-3-a. Type 1. Genetic modifications with nucleic acid from any source that result in organisms that are no longer able to express a normal trait considered hazardous to human health or to managed or natural ecosystems"; and, change existing Type 1 (II-B-3-a) to Type 2; Type 2 (II-B-3-b) to Type 3; and Type 3 (II-B-3-c) to Type 4.

Section III. Accept, with the correction on page 8 in Section III-B, next to last line, change "provided' to "provide."

Section IV. Accept, with modifications and exact language of changes to be developed, and to include the addition of Section IV-A-5 for Class 5 experiments with Status 5 organisms, and designation of classes of experiments under appropriate headings in IV-B.

Section V. Accept, with the following modifications:

In Section V-A-2 (p. 13), change IV-B-1 to V-B-1, and IV-B-2 to V-B-2; and

In Section V-B-2-b (p. 15), line one, insert the word "may" after IBC; and on line four, delete the word "not" both times it occurs.

Section VI. Accept.

Section VII. Accept the draft test placed on the table today, with the modification of replacing the word "petition" in four places with the word "request."

Section VIII. Accept, with typographical changes: on p. 20, change VIII-2 to VIII-B; and on p. 21, in five places, change references to Section IV to Section V. These are at the end of VIII-B-2, VIII-B-4, two places in VIII-B-5, and at the end of VIII-D-3.

Dr. Frey expressed concern about the language in the section describing the purpose of the guidelines. He specifically wished to avoid review by ABRAC if a regulatory agency had already reviewed and approved a particular proposal. Dr. Vidaver referred to language in the NIH Guidelines waiving NIH review if a proposal has been reviewed by another Federal agency. Dr. Frey suggested specific language to accomplish that purpose in the USDA guidelines. Dr. Kemp expressed the view that the specific language suggested by Dr. Frey added an inconsistency to the guidelines. Dr. Korwek added that

appropriate language exempting proposals reviewed by another agency from ABRAC review is feasible. Dr. Osburn requested that Dr. Korwek draft such language.

Dr. Osburn turned the Committee's attention to Section V of the draft guidelines on roles and responsibilities, including those of Institutional Biosafety Committees (IBC's). Committee members raised various questions about membership requirements, accident reporting, conflict of interest, and record-keeping requirements of IBC's. Dr. Osburn asked Ms. Hollander to work with Dr. Gould and Dr. Gorham to review alternative language relating to IBC's and to develop recommendations for the Committee.

Dr. Osburn turned the Committee's attention to a part of Dr. Tolin's motion to accept the December 5, 1988 with the changes she indicated. That part of the motion included Sections VI - Protection of Proprietary Data, Section VII - Amendment Procedures, and Section VIII - Definitions. Dr. Serdy expressed concern about the absoluteness of the definition of safety in Section VIII. [That definition was "the prevention of unreasonably adverse effects on human health or on managed or natural ecosystems."] Dr. Tolin replied that the definition in question was the recommendation of the Guidelines Working Group and that it could be changed by the Committee.

A Committee member moved to call the question. The Committee voted 8 to 4 with 1 abstention to terminate debate on Sections VI, VII. and VIII. Dr. Osburn called for a vote on accepting those sections of the December 5, 1988 draft with the changes listed by Dr. Tolin. The Committee voted 7 to 5 with 1 abstention to accept those sections with the indicated changes.

Dr. Osburn turned the Committee's attention to the remaining portion of Dr. Tolin's motion. That portion included Sections II - Classification of Organisms, Section III - Confinement Principles, and Section IV - Conduct and Review of Experiments.

Dr. Osburn invited discussion on Section II on Classification of Organisms. Dr. Korwek raised an objection to the limitations on methodologies that are within the scope of the guidelines in Section II-B-1. He questioned the purpose of a broad definition of biotechnology if a more limited definition actually applies. Dr. Whitmore indicated that the limitations were included in order to exclude traditional technologies from the scope of the guidelines. Dr. Phaire-Washington and Dr. Vidaver expressed support for excluding traditional technologies. Dr. Gould expressed support for Dr. Korwek's view. Ms. Hollander recommended retention of a broad definition of biotechnology to be more consistent with other agencies. Dr. Korwek recommended that the guidelines define biotechnology and the scope of the guidelines broadly, and then list exempted methods including traditional ones in Section II-B-3 under Type 1.

Dr. Osburn invited discussion on Section III on Confinement Principles. Dr. Gould expanded on his concerns about the confinement approach described in his letter of November 16, 1988 (Appendix G). After some further discussion, Dr. Osburn asked Drs. Gorham, Purchase, Gould, and Sorensen to review the confinement section and draft changes reflecting the sense of the Committee. Dr. Osburn recessed the meeting at 4:40 p.m.

BRUCELLOSIS RESEARCH PROPOSAL FROM TEXAS A&M UNIVERSITY

Dr. Osburn reconvened the Committee at 9:10 a.m. on January 6, 1989. He asked Dr. Gorham, Chair of the Confinement Working Group, to introduce the brucellosis research proposal from Texas A&M University. [The Committee had previously been supplied with an OAB working paper on the Texas A&M proposal (Appendix H)]. Dr. Gorham summarized the main points of the proposal and introduced Dr. L. Garry Adams, the Principal Investigator for the Texas A&M proposal.

Dr. Adams gave the Committee an oral presentation on the Texas A&M brucellosis research proposal. He indicated the need for an improved vaccine for brucellosis in cattle. He reviewed the history of the project and provided a technical description of the genetic construct. He said the test organism is a Tn5 transposon mutant of Strain 19 of *Brucella abortus*, the causative agent of brucellosis in cattle. The transposon mutant was prepared by the method of Smith and Heffron whose published paper had been supplied previously to members of the Committee. In laboratory culture, colonies of the transposon mutant exhibit the "rough" phenotype as opposed to the "smooth" phenotype of the virulent Strain 2308 commonly used to conduct immunological challenges of vaccinated animals. Dr. Adams displayed maps and aerial views of the test site and he described the physical, chemical, and biological barriers that he intended to use to confine the test organism to the test site.

Dr. Kemp asked if the genetic change in the mutant strain was known to be a deletion or if it could also be an insertional inactivation. Dr. Adams acknowledged that the genetic change could be either a gene deletion or an insertional inactivation. Dr. Korwek asked if the mutant strain was recombinant. Dr. Kemp replied that it was because of the Tn5 insertion.

Dr. Phaire-Washington asked what procedures are followed to monitor possible reversions of the mutant strain in cattle. Dr. Adams replied that antibody assays are run weekly so that a reversion to the smooth phenotype would be detected within a week. Dr. Kemp asked if Dr. Adams had ever seen a reversion of the mutant strain. Dr. Adams replied that he had not. Dr. Whitmore asked if a reversion would be different from a natural infection. Dr. Adams replied that a reversion would not differ from a field strain infection.

Dr. Osburn temporarily delegated the responsibilities of the Chair to Dr. Young so that Dr. Osburn could devote his full attention to a scientific commentary on the Texas A&M proposal.

Dr. Gorham distributed the written recommendation of the Confinement Working Group (Appendix I) to the Committee members. The recommendation read as follows: "The biosafety and confinement conditions for the <u>Brucella</u> research proposed by Texas A&M will be adequate to ensure no unreasonable risk to human safety and the environment if the additions and clarifications listed below are added to the protocol. With these additions and clarifications, the biosafey provisions will provide a level of protection greater than that which occurs naturally in an infected area."

Dr. Gorham moved that the Committee accept the proposed Texas A&M confinement procedures, with certain additions and clarifications, as scientifically adequate to conduct the test safely.

Dr. Gorham enumerated the following additions and clarifications for the test procedures as proposed by Texas A&M. The tetracycline-sensitivity of the Tn5 transposon mutant should be maintained throughout the test. A full face mask respirator should be worn by researchers while conducting immunological challenge with highly infectious materials. A pulsating high-voltage electric fence should be constructed around the test site to discourage intrusion of non-test animals. The test should be scheduled, to the extent possible, so that <u>ex vivo</u> survival and movement of the test and challenge organisms are limited by seasonal weather conditions. Sentinel animals should be distributed around the periphery of the test site and, if they seroconvert, they should be slaughtered and cultured immediately with appropriate notification of the Institutional Biosafety Committee and APHIS. Good agricultural practices and appropriate physical environmental, chemical, and biological barriers should be used at all times during the test.

Dr. Young invited Dr. Shibley of the Animal and Plant Health Inspection Service (APHIS) to comment on the Texas A&M research proposal. Dr. Shibley expressed his appreciation for the invitation to participate in the working group that addressed the Texas A&M proposal. He briefly described the APHIS review procedure and indicated that if the Texas A&M petition to be submitted to APHIS is in accordance with the regulations, then the trial would be authorized.

Dr. Young invited Mr. Barbeito of the Agricultural Research Service (ARS) to comment on the Texas A&M proposal. Mr. Barbeito indicated that he had discussed the etiologic classification of this organism with representatives of the Centers for Disease Control (CDC). He indicated that the CDC representatives were not receptive to changing the classification of the organism. Instead, he expressed the view that, from

a biosafety perspective, it may be more realistic to submit the proposal to APHIS for an evaluation of the animal health and environmental effects. Mr. Barbeito concluded with an expression of support for the development of an improved vaccine to solve a major disease problem of the U.S. livestock industry.

Dr. Young invited Dr. Osburn to comment on the Texas A&M proposal. Dr. Osburn noted evidence that the Tn5 transposon mutant exhibits reduced pathogenicity compared to the parent strain. He observed that many veterinarians besides himself are commonly exposed to the virulent Brucella organism in the course of their research and that they are highly motivated to handle the collection and disposition of infected tissues carefully. The remaining question to be addressed, in his view, was whether the mutant organism will serve as an effective vaccine in the host animals. Dr. Osburn expressed the belief that the confinement procedures proposed by Texas A&M and expanded upon by the Confinement Working Group are adequate to conduct the test safely. He concluded with an expression of support for the motion before the Committee.

Dr. Young invited discussion from the full Committee. Dr. Gould made a reference to Dr Adams' earlier estimate of approximately 1000 infected herds as the current prevalence of naturally-acquired brucellosis in Texas. Dr. Gould asked if the worst case scenario from the proposed test could be envisioned appropriately as 1001 infected herds instead of 1000. Dr. Adams agreed in general with that projection, but he qualified it with a technical reservation concerning the length of the infection.

Dr. Kemp asked if there are any facilities other than the special facilities at Plum Island, New York, to do this kind of research. Mr. Barbeito expressed the view that there are none. Dr. Thiermann of ARS contrasted the limited space at Plum Island intended for exotic animal disease work with the wide prevalence of brucellosis in 1000 herds in Texas. He expressed the view that the environmental risks are adequately addressed in the Texas A&M proposal and that the human risks can be handled adequately in ways other than confinement to Plum Island.

Dr. Demain asked Dr. Adams for his view on the additional confinement measures recommended by the Confinement Working Group. Dr. Adams indicated that he could implement all of the recommended confinement measures with the exception of the fully enclosing chain-link fence and the full-time surveillance. Dr. Adams offered to substitute electronic fencing developed in Australia to discourage intrusion of wild carnivores such as dingos. He said his research group has had success with such electronic fencing as a deterent to coyotes. Dr. Adams stated that his institution would have great difficulty in providing facilities and resources for someone to live on the premises full-time. He indicated that such a person would represent a potential disease exposure liability and he preferred that only

professional veterinarians be exposed to such risks. As an alternative to full-time manual surveillance, Dr. Adams offered to implement an electronic surveillance system that had already been requested by his Institutional Biosafety Committee.

Dr. Kemp moved to amend Dr. Gorham's motion by incorporating the modifications suggested by Dr. Adams. Ms. Hollander requested a clarification of which confinement measures Texas A&M is already practicing. Dr. Adams indicated that his team has implemented all the confinement measures except the use of full face mask respirators, chain-link fencing, and full-time manual surveillance. Dr. Adams agreed to the use of full face mask respirators and he had already proposed the alternative measures of electronic fencing and electronic surveillance. Ms. Hollander proposed addition of the phrase "recommended additions and clarifications regarding biosafety" to the description of the confinement procedures.

A Committee member called for the question on the amendments. The Committee voted 12 to 0 with 1 abstention to accept Dr. Kemp's motion to incorporate the amendments offered by Dr. Adams into the Working Group recommendation. A Committee member called for the question on the Working Group recommendation. The Committee voted 12 to 0 with 1 abstention to accept the Working Group recommendation that the Committee accept the proposed Texas A&M confinement procedures, with certain additions and clarifications, as scientifically adequate to conduct the test safely. Dr. Young indicated that OAB would prepare appropriate correspondence for the Assistant Secretary. He then returned responsibility of the Chair to Dr. Osburn.

NBIAP ACTIVITIES

Dr. Osburn asked Dr. MacKenzie to update the Committee further on the NBIAP. Dr. MacKenzie indicated that his staff has completed a strategic plan for the program and developed three different scenarios depending on the level of funding that is attained. He described discussions with a major university to access various biological databases through the BITNET computer network. He also described a recent meeting jointly sponsored by USDA and the Agricultural Research Institute on plant genome mapping.

RESEARCH GUIDELINES

Dr. Osburn asked Dr. Korwek to report on the progress of the drafting group on the purpose and applicability of the guidelines. Dr. Korwek presented the following revised text for Section I - Purpose: "These guidelines are designed to specify practices and procedures for agricultural research outside the laboratory involving biotechnology. The guidelines establish fundamental principles upon which specific experiments and organisms can be evaluated and upon which confinement practices can be based." Dr. Korwek also proposed the following

language for Section II - Applicability: "These guidelines apply to all biotechnology research which is conducted at, or sponsored by, an institution or performed by persons receiving any support from USDA."

Dr. Korwek proposed the following language for Section II relating to review by other agencies. "Once approval or other applicable clearances have been obtained from such federal agency, the experiment may proceed without ABRAC review. Institutions and persons conducting biotechnological research outside the laboratory that is not funded by USDA or that is not subject to review by ABRAC are encouraged to comply with applicable portions of the guidelines on a voluntary basis."

Dr. Hollinshead proposed alternate language for Section II on Purpose [Appendix J].

After extensive discussion by Committee members and others, Dr. Korwek moved that the Committee adopt his proposed text for Sections I and II with revisions reflecting the sense of the Committee. Dr. Korwek's motion carried unanimously.

Dr. Osburn asked Ms. Hollander to report on the progress of the drafting group on Section V - Roles and Responsibilities [Appendix K]. Ms. Hollander described a number of suggested changes to that Section including the possible mandatory nature of the guidelines, OAB monitoring of IBC membership, accident reporting requirements, newspaper announcement of IBC meetings, financial interests of IBC members, and confidential business information. Ms. Hollander moved that the suggested changes be adopted by the Committee.

Dr. Osburn opened the floor to discussion. Committee members noted the controversial nature of several of the suggested changes and expressed specific reservations about some of them. Dr. Korwek noted the difficulties of institutional acceptance of newspaper announcement of IBC meetings. Dr. Frey disagreed with the implication that receipt of a salary from a company should disqualify an expert from serving on the company IBC. One member called the question. The Committee voted 7 to 3 with no abstentions not to adopt the suggested changes in Section V.

Dr. Tolin moved to accept Section V as it appeared in Tab 72 with changes in accordance with the sense of the Committee. The motion carried unanimously.

Dr. Osburn asked Dr. Gorham to summarize suggested changes in Section III - Confinement Principles. Dr. Gorham enumerated several changes in that Section [Appendix L]. A member called the question and the Committee voted unanimously to adopt the changes suggested by Dr. Gorham.

Dr. Osburn turned the Committee's attention to Section II - Classification of Organisms and Section IV - Conduct and Review

of Experiments. Dr. Tolin described a number of suggested changes to those sections worked out by herself, Mr. Stern, and Drs. MacKenzie, Purchase, and Whitmore during the lunch break [Appendix M]. Dr. Frey also proposed new language for Section II [Appendix N]. Committee members expressed diverse views on them. Dr. Frey moved that OAB prepare a new version of the guidelines based on the sense of the Committee and recirculate it for Committee review. The motion carried unanimously.

Dr. Young requested authorization from the Committee for OAB to prepare a revised version of the entire document. Dr. Osburn requested a show of hands and the Committee voted 8 in favor, 1 opposed, with 0 abstentions to authorize OAB to revise the whole guidelines document.

Dr. Tolin moved to adjourn and Dr. Osburn adjourned the meeting at 3:40 p.m.

Daniel D. Jones

Daniel D. Jones, Ph.D.
Rapporteur

Alvin L. Young

Alvin L. Young, Ph.D.
Executive Secretary

Bennie I. Osburn

Bennie I. Osburn, D.V.M., Ph.D.
Chair

LIST OF APPENDICES

A. Members, USDA Agriculture Biotechnology Research Advisory Committee, January 5-6, 1989

B. Summary of Guideline Working Group Recommendations

C. Summary of Recommendations for Research Outside the Laboratory and Sederoff letter of December 3, 1988

D. Whitmore memorandum dated January 2, 1989 on guideline revisions

E. Stern memorandum dated December 14, 1988 on guideline revisions

F. MacKenzie handout on Classification of Organisms

G. Gould letter of November 16, 1988 on guideline revisions

H. OAB Working Paper on Texas A&M Proposal for Research with Live Recombinant *B. abortus* Outside the Laboratory

I. Recommendation of the USDA Agricultural Biotechnology Research Advisory ommittee (ABRAC) on the Texas A&M Brucellosis Biosafety Research Proposal, January 6, 1989

J. Hollinshead Draft, 1/6/89

K. V. Roles and Responsibilities, Draft of Hollander's Group

L. III. Confinement principles, Draft of Gorham's Group

M. II. Classification of Organisms, Draft of Tolin's Group

N. II. Factors affecting safety of biotechnology research outside the laboratory, Frey's draft

MEMBERS

UNITED STATES DEPARTMENT OF AGRICULTURE

AGRICULTURE BIOTECHNOLOGY RESEARCH ADVISORY COMMITTEE

January 5-6, 1989

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SUMMARY OF GUIDELINE WORKING GROUP RECOMMENDATIONS

Chronology

Sept. 22-23 ABRAC Meeting. Major revisions suggested.

Oct. 24 Annotated Version of Sept. 6, 1988 Draft with changes made to reflect ABRAC suggestions. Circulated to ABRAC members for comment.

Nov. 30 Working Draft (without annotations)

Dec. 2 Working Group meeting -
Discussions of changes from ABRAC and comments.
Order of discussion:

I. Purpose
Major revisions by vote and consensus.
See suggestion from Stern (Doc. 73).

V. Roles and Responsibilities
Changes made to reflect ABRAC input.

VI. Protection of Proprietary Data
No change - see handbook.

VII. Amendment Procedure
Additional details needed:Jones/Stern.

VIII. Definitions
Modified "managed or natural ecosystems"
Added "safety"; deleted "organism".

II. Classification of Organisms
and
IV. Conduct and Review of Experiments

Discussed; major changes made, although consensus not reached. Needs ABRAC input. See Stern (Doc. 73) for II-A.
In II, delete reference to Appendix A.
In IV-A, insert "Modified" before Organism. Needed to utilize II-B-3.
In IV-A, change Status to Category, which equals Status x Type Modification.
See Sederoff's Summary of Recommendations:

III. Confinement
Did not discuss because of time limit.

Dec. 5 Draft Guidelines distributed to ABRAC for January 5-6.
Jan. 5 Sederoff Summary Table.
Whitmore Summary Table.

APPENDIX C

SUMMARY OF RECOMMENDATIONS
FOR RESEARCH OUTSIDE THE LABORATORY

II-A STATUS OF ORGANISM	II-B3 TYPE OF MODIFICATION	IV-A CATEGORY OF MODIFIED ORGANISM	III-C CONFINEMENT LEVEL	IV-B LEVEL OF REVIEW
1	1	1	1	IBC Notice
1	2	1	1	IBC Notice
1	3	2	2	IBC Approval
2	1	1	1	IBC Notice
2	2	2	2	IBC Approval
2	3	3	3	IBC and USDA Approval with ABRAC Review
3	disarmed	1	1	IBC Notice
3	1	2	2	IBC Approval
3	2	3	3	IBC and USDA Approval with ABRAC Review
3	3	4	4	IBC and USDA Approval with ABRAC review
4	disarmed	2	2	IBC Approval
4	1	3	3	IBC and USDA Approval with ABRAC review
4	2	4	4	IBC and USDA Approval with ABRAC review
4	3	5	inside the laboratory	—
5	disarmed	3	3	IBC and USDA Approval with ABRAC Review
5	1	4	4	IBC and USDA Approval with ABRAC Review
5	2	5	inside the laboratory	—
5	3	5	inside the laboratory	—

Formulated by Dr. Ronald R. Sederoff, North Carolina State University, December 20, 1988.

TOTAL P.02

North Carolina State University

Department of Forestry

School of Forest Resources
Box 8002, Raleigh 27695-8002

3 December 1988

Alvin L. Young, Director
Office of Agricultural Biotechnology
Office of the Secretary
US Department of Agriculture
Washington DC, 20250

Dear Dr. Young,

I am writing to reply to your request for a brief summary and analysis of the meeting of the ABRAC guidelines subcommittee on 2 December 1988. Writing of the guidelines is well under way. The overall logical structure of the guidelines has been created and is firming up well. Several major problems have been resolved, but much work remains to be done in finding the best language and format.

The subcommitte has reviewed and revised several sections after considering comments from other ABRAC members, OAB staff, and concerned individuals. Major expansion and revision was done with respect to the section on classification of genetic modifications. With the new section on levels of containment, it remained to develop a strategy for the "marriage" of the sections on status of organism, type of modification, and level of containment.

The procedure that the subcommittee is using as a working strategy is as follows. The status of the organism will be established based on the existing knowledge of the properties of the organism, based on principles that will be explained and with examples. The modification of the genetic composition of the organism will be considered based on the knowledge of the properties of that modification, also with examples. The modification will then raise or lower the status of the organism according to instructions and examples in a table. The table will assign the modified organism to a category which will establish the recommended level of confinenent. The PI will then choose the appropriate methods for the achievement of confinement. One category of experiments will be exempt from confinement, other will need advance approval from the IBC, and some experiments will require approval of the ABRAC.

If the most important task of the committee is to figure out what it is we have to say to have workable guidelines, then we have made great progress. It is the first time that we have figured out what the guidelines will contain and how they will work. Having finally figured out what to say, we now have only to figure out how best to say it. No doubt, many drafts and revisions will be needed as the guidelines take shape and they become subject to more intense levels of scruitiny.

We should be pleased that the guidelines are taking shape in a way that is based on biological principles, that is, the guidelines will be based on the properties of the organism, the biological properties of the modification, and can be applied to any experiment with any organism. If the guidelines are scientifically sound, they will be easy to defend to anyone as appropriate to protect the environment, to facilitate research in the private sector, to unencumber academic research, and to fulfill the regulatory role of government. Guidelines firmly established on available knowledge and consistent fundamental principles will have the strongest possible position if they are challenged in a court of law. We are moving slowly, but deliberately in the right direction.

Thank you for the invitation to attend this meeting.

Sincerely,

Ron Sederoff

Ron Sederoff
Professor of Forestry and Genetics

Subject USDA Guidelines for Research Outside the Laboratory Involving Biotechnology

Date January 2, 1989

From Bill Whitmore

To Sue Tolin, Chair, Guidelines Working Group

THE OHIO STATE UNIVERSITY

COMMUNICATION

Here are several revisions of the Guidelines draft that have occurred to me during a review over the holidays.

II-A Classification of organisms.

I like the revisions made by Paul Stern in his memo to Al Young of December 14, 1988. I would make the further suggestion:

> Reduce the Statuses of organisms to four. Combine Status 3 and 4.

I think that the distinction between "adverse effects" and "serious adverse effects is not sufficient to make a separation, and would present a difficulty for an investigator. Since so much of this is prediction, it would simplify things to combine these two statuses. Another argument for this combination is the probability that all experiments with organisms higher than Status 1 or 2 will come to the ABRAC in the early stages of the system. Status 4 would be reserved for the most troublesome organisms that would not be allowed outside the lab.

II-B-3. Types of genetic modification.

In this section, I suggest adding another type, as follows:

> II-B-3-a. Type 1. Genetic modifications with nucleic acid from any source that result in organisms that are no longer able to express a normal trait considered hazardous to human health or to managed or natural ecosystems (such normal traits that cause the unaltered organism to be placed in a Status higher than Status 1).
>
> II-B-3-b. Type 2. (Same as Type 1 in December 5 draft)
>
> II-B-3-c. Type 3. (Same as Type 2 in December 5 draft)
>
> II-B-3-d. Type 4. (Same as Type 3 in December 5 draft)

The rationale for this change is that the new Type 1 would be the opposite of the original Type 3 (now Type 4) in terms of biological or ecological effect. Probably more experiments would make use of this new Type 1 modification than the original Type 3. In fact, the Brucella experiment proposed by Texas is just such a case, at least in reference to the live transposon mutant vaccine. This Type 1 would also introduce a sort of negative value that could, in some cases, lower the effective status of the test organism. It may also add some esthetic balance to the original Type 3.

As an exercise, I have taken a crack at using these revisions in a table:

ORGANISM	GENETIC MODIFICATION	ORGANISM	CONFINEMENT	REVIEW
Original Status	Type	Resulting Status	Level	Approval by:
1	1	1	1	IBC
1	2	1	1	IBC
1	3	2	2	IBC and USDA
1	4	3	3	IBC and USDA
2	1	1	1	IBC
2	2	2	2	IBC and USDA
2	3	2	2	IBC and USDA
2	4	3	3	IBC and USDA
3	1	2	2	IBC and USDA
3	2	3	3	IBC and USDA
3	3	3	3	IBC and USDA
3	4	4	4	IBC and USDA

PAUL ELIHU STERN, ESQUIRE
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December 14, 1988

MEMORANDUM

TO: Alvin L. Young, Director, OAB

FROM: Paul Elihu Stern

SUBJECT: USDA Guidelines for Research Outside the Laboratory Involving Biotechnology

After reflecting on the December 2, 1988, ABRAC Working Group meeting on the Guidelines and discussing the issues with you, Ron Sederoff, and Sue Tolin, I have developed the enclosed document. The discussion presents some proposed alternative language for the Guidelines with accompanying explanation. The major point is that the Guidelines are not being developed to prevent harm, because no harm has been specifically identified that is associated with research outside the laboratory involving biotechnology. There are, however, uncertainties and public concerns, which should be addressed. These uncertainties and concerns can be addressed through Guidelines which promote safe experimentation. Thus, the Guidelines should not be designed to "prevent unreasonable adverse effects" of experiments, but to promote experiments which are "compatible with the environment."

December 14, 1988

Proposed language for Section I:

PURPOSE: These Guidelines are designed to specify practices and procedures to promote agricultural research outside the laboratory involving biotechnology which is compatible with the environment. Research outside the laboratory which is compatible with the environment is achieved by limiting the potential for (1) adverse effects on human health or on managed or natural ecosystems, (2) transfer of genetic material, (3) rapid and widespread dissemination, and (4) uncontrollable reproduction of organisms used in research outside the laboratory. The Guidelines establish fundamental principles upon which the safety of specific experiments and organisms can be evaluated and upon which confinement practices can be based.

Explanation of Section II-A of the October 24, 1988, draft:

In analyzing the October document, one should go back to the minutes of the Working Group meeting during the September ABRAC meeting. First, three areas of concern were identified: transfer, dissemination, and reproduction. Then, the concept of pathogen was introduced, and it was determined that pathogenicity, in itself, was not a good criterion upon which to judge safety. Based on that, the notion of "unreasonable adverse effects" seemed to fit well. That is, adverse effects, alone, are not bad; the entire results of a particular experiment must be examined. Therefore, an experiment without unreasonable adverse effects (equivalent to an experiment of "predictably low consequence to the environment") is what we are pursuing. As noted on page 10 of the October 24 draft, OAB was troubled by the potential ambiguity of the terms, "pest or pathogen," "low potential," "generally recognized as having a predictable low consequence," "moderate consequence," and "high consequence."

Thus, the October draft is an attempt to reduce potentially ambiguous language.

With that background, if you look at Status 1, set out on pages 7 and 8 of the October draft, an experiment is generally recognized as compatible with the environment (GRACE) that has no recognized potential for unreasonable adverse effects or for transfer of genetic material or for rapid and widespread dissemination or for uncontrollable reproduction.

Status 2, then, is also "low consequence," but there are potentials for unreasonable adverse effects or for transfer of genetic material or for rapid and widespread dissemination or for uncontrollable reproduction. This category also covers the situation where an organism might have the potential for transfer of genetic material or for rapid and widespread dissemination or for uncontrollable reproduction, but it does not cause unreasonable adverse effects. (This case was discussed by the confinement working group. The group felt there was little need for confinement, if the organisms would not cause harm.)

Status 3 was designed to cover the "moderate consequence" organisms. Thus, not only do these organisms have a recognized potential for unreasonable adverse effects, but they also can transfer genetic material or disseminate rapidly and widely or reproduce uncontrollably. The examples, then, show that the consequence is not severe.

Status 4 includes the "high consequence" organisms. If they transfer genetic material or disseminate rapidly and widely or reproduce uncontrollably, the resulting harm will be more than unreasonable. These are organisms that are very difficult to control.

Finally, Status 5 describes those organisms which present such grave danger that they should only be handled in contained laboratories.

<u>Discussion of the December 2, 1988, Working Group meeting on Guidelines</u>:

At the December 2 meeting of the Working Group on the Guidelines, the use of the term "unreasonable" caused much apprehension among the members. OAB has therefore proposed language which does not utilize that term. That approach fits well with the idea that the GRACE concept should be utilized throughout, so that all experiments in compliance with the Guidelines are compatible with the environment. Status 1 organisms with no confinement are GRACE. Status 5 organisms are never GRACE. Status 4 organisms with the highest confinement level are also GRACE. And Status 2 and Status 3 organisms are GRACE when combined with confinement levels from the lowest to the highest level.

The Working Group also discussed at the December 2 meeting the meaning of extensive knowledge. The extremes are not difficult to show, but the middle is more difficult. This is why we have IBCs and ABRAC. For the most controversial experiments or those with organisms about which little is known, ABRAC would evaluate the potential for unreasonable adverse effects, the potentials for transfer of genetic material, for rapid and widespread dissemination, and for uncontrollable reproduction. As knowledge and experience accumulate, decisions can be delegated to IBCs and, ultimately, to PIs.

"Recognized potential" or "generally recognized" should be equated with extensive knowledge. We will have a hard time justifying the conclusions of the Guidelines based on much less. That does not mean that everything about

an organism must be known before field research is conducted, but a considerable amount should be known about its potential for transfer of genetic material or rapid and widespread dissemination or uncontrollable reproduction.

Based on the above discussion, please consider the following proposed language for Section II-A:

STATUS 1. Organisms which are generally recognized as compatible with the environment (GRACE). These organisms have —

(a) **little or no recognized potential** for adverse effects on human health or on managed or natural ecosystems **and**

(b) **little or no recognized potential** for (1) transfer of genetic material or (2) rapid and widespread dissemination or (3) uncontrollable reproduction.

STATUS 2. Organisms which have—

(a) **recognized potential** for adverse effects on human health or on managed or natural ecosystems **or**

(b) **recognized potential** for (1) transfer of genetic material or (2) rapid and widespread dissemination or (3) uncontrollable reproduction.

(If an organism has the potential for adverse effects but cannot transfer genetic information, disseminate rapidly or widely, or reproduce uncontrollably, its adverse effects are not unreasonable and the consequences are, therefore, predictably low.)

STATUS 3. Organisms which have—

(a) **recognized potential** for adverse effects on human health or on managed or natural ecosystems **and**

(b) **recognized potential** for (1) transfer of genetic material or (2) rapid and widespread dissemination or (3) uncontrollable reproduction.

(If an organism has the potential for adverse effects and can transfer genetic information, disseminate rapidly or widely, or reproduce uncontrollably, its adverse effects are unreasonable, and the consequences are, therefore, predictably moderate. By itself, this may not hold completely true, because one might suggest that the consequences could be greater than moderate, but, with STATUS 4, it makes sense.)

STATUS 4. Organisms which have—

(a) **recognized potential** for **serious** adverse effects on human health or on managed or natural ecosystems **and**

(b) **recognized potential** for (1) transfer of genetic material or (2) rapid and widespread dissemination or (3) uncontrollable reproduction.

(If an organism has the potential for serious adverse effects and can transfer genetic information, disseminate rapidly or widely, or reproduce uncontrollably, its adverse effects are more than unreasonable, and the consequences are predictably high.)

STATUS 5. Organisms which have—

(a) **recognized likelihood** for **serious** adverse effects on human health or on managed or natural ecosystems **and**

(b) **recognized likelihood** for (1) transfer of genetic material or (2) rapid and widespread dissemination or (3) uncontrollable reproduction.

(This is our keep-it-inside category.)

Discussion of possible application of proposed language:

At this point, the remainder of the Guidelines can be structured. Using the points developed in the genetic modification section, an initial evaluation should be made about the organism to be utilized in the proposed experiment. First, from which status does the nonmodified organism come? Second, what genetic modification(s) was/were made to that organism? Third, what was the effect of that/those modification(s) on the potential of the organism for unreasonable adverse effects or transfer of genetic material or rapid and widespread dissemination or uncontrollable reproduction? If the genetic modification(s) has/have changed the potential of the organism for any of the above, there may be a status change. If the potential for adverse effects or for transfer of genetic material, or for rapid and widespread dissemination, or for uncontrollable reproduction has not changed, the modified organism is then equivalent to a nonmodified organism of the initial status determination.

The confinement section does need some work, but if it is keyed to the potential of the particular organism for adverse effects, for transfer of genetic material, for rapid and widespread dissemination, and for uncontrollable reproduction, we will have a tight document.

The requirements for review could be tied to change in status as well as to status, itself. Then, we are not basing the Guidelines on whether the

organism is genetically engineered, but on the character of the organism. From the point of view presented in the "Coordinated Framework," the Guidelines will be product-based rather than process-based.

11-A. Classification of organisms.

11-A-1. Status 1. Organisms generally recognized as compatible with the environment (GRACE). Status 1 organisms have little or no recognized potential for transfer of genetic information to other organisms, or for rapid and widespread dissemination in the environment, or for uncontrollable repoduction, or for unreasonable adverse effects on human health and on managed or natural ecosystems, such as, domesticated organisms and organisms with a long history of use.

11-A-2. Status 2. Organisms with potential (a) for unreasonably adverse effects on human health or on managed or natural ecosystems and (b) for transfer of genetic information to other organisms, or for rapid and widespread dissemination in the environment, or for uncontrollable reproduction. Examples of Status 2 organisms are gypsy moth, kudzu, southern corn leaf blight pathogen, and water hyacinth.

11-A-5. Status 3. Organisms with (a) great potential for serious harm to human health or to managed or natural ecosystems and (b) great potential for transfer of genetic informaation to other organisms, for rapid and widespread dissemination in the environment, or for uncontrollable reproduction, such as, exotic organisms. Examples of Status 3 organisms are striga foot and mouth disease virus, plum pox virus and soybean rust fungus.

11-B-3. Types of genetic modification.

11-B-3-a. Type 1. Genetic modification with recombinant DNA technology (_sensu_ NIH-RAC guidelines) that have previously undergone NIH review and approval, IBC review and approval or are exempt from the NIH guidelines for contained experimentation with recombinant DNA.

11-B-3-b. Type 2. Genetic modifications by biotechnology other than recombinant DNA technology (_sensu_ NIH-RAC guidelines).

Level of Review Required

Organism Classification

Type of Modification	Status 1	Status 2	Status 3
1	IBC Review and ABRAC Notification	ABRAC Review	ABRAC Review
2	ABRAC Review	ABRAC Review	Field Tests Not permitted at this time

Probable Confinement Level

Organism Classification

Type of Modification	Status 1	Status 2	Status 3
1	1	2	4
2	1	3	Not permitted

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16 November 1988

Dr. Alvin Young
O.A.B., USDA
Room 321-A
Washington, D.C. 20250-2200

Dear Al:

The following are my comments on the revised guideline:

Page 7 - II A-1 Status 1

a) *transfer of genetic information to other species or transfer to pest or pathogenic races of the same species (organism is an ambiguous term).

c) Does rhizobium fit here? Corn only fits in some places, not Mexico.

II A-2 Status 2

a) Transfer of genetic

II A-4 - II A-5

This is a continuum - where does gypsy moth fit? If we cannot come up with good unambiguous examples, we may get into trouble.

Page 9 - lines 9-10. If we do not include "introduction solely of same-species DNA or RNA," in our definition then categories II B-3a and II B3b are not generally covered.

Since genomic rearrangements of same species DNA or insertion of multiple copies can change expression levels in ways that natural breeding cannot, where are we?

Also, what happens when you take the promotor sequence of one gene in plant A and fuse it with the coding region of another gene of plant A? The guidelines would not cover this, but it may become common.

Page 9, section II B2, lines 6-8. Given this wording, it is not apparent why we think we need guidelines at all. I think we will get shot out of the water on this one.

Page 10, II B-3c (1-3). Take the words "or genetic" out.

Page 11, 4 lines from bottom. Replace <u>and</u> with <u>or</u>.

Page 11, 2 lines from bottom. Take <u>unreasonable</u> out.

Page 19, III-C-1, level one. Also include off-site practices including transfer of seed from the lab, etc. Generally this equals no confinement.

I generally find this approach to level of confinement bothersome. The bottom line is that we need to deal with the number of individual escapes of a particular genotype that we think is O.K. Then we need to require an approach to testing that will keep us below that number. (I know it's not easy, but this other approach seems too vague and open to constant challenge.)

These are rough notes. I'd be happy to discuss them in more detail over the phone.

Sincerely,

Fred

Fred Gould
Associate Professor
Entomology

FG/cs

OAB Working Paper on Texas A&M Proposal for Research with Live Recombinant B. abortus Outside the Laboratory

BACKGROUND

On April 18, 1988, the Office of Agricultural Biotechnology (OAB) received a letter from Dr. L. G. Adams at the Texas Veterinary Medical Center, Texas A&M University, requesting guidance and approval for research in cattle using live recombinant Brucella abortus, the causative agent of brucellosis in cattle, at a containment level lower than National Institutes of Health (NIH) Biosafety Level 3N (BL-3N). The proposal is provided in Attachment 1.

The Texas A&M Institutional Biosafety Committee (IBC) and the Office of Recombinant DNA Activities at NIH approved research to evaluate a promising new vaccine (Brucella abortus, class 3, USDA strain 19, containing Tn 5 transposon, rough mutants) in mice and goats under NIH BL-3N conditions. This work has been completed and the agent is now ready to be evaluated in cattle.

OAB has also received requests for guidance from two other institutions planning research with recombinant B. Abortus (Agricultural Research Service, National Animal Disease Center, Ames, IA and Scripps Research Institute, LaJolla, CA); however, specific proposals have not been submitted by these institutions.

Anticipated Step-Wise Process of Review

The Agricultural Biotechnology Research Advisory Committee (ABRAC) has been requested to review the scientific merits of the biosafety provisions in the Texas A&M proposal and advise USDA's Assistant Secretary for Science and Education on whether this experiment conducted outside the laboratory can meet sufficient field confinement conditions to ensure safety and protection of the environment. USDA funding of the study is involved; therefore, it should be conducted consistent with the scientific principles under consideration by ABRAC for the "USDA Guidelines for Research Outside the Laboratory Involving Biotechnology." If the recommendation from ABRAC is affirmative and is acceptable to the Assistant Secretary, Texas A&M will be so advised.

OAB then will assist Texas A&M in applying for a waiver from the Department of Health and Human Services Communicable Disease Center (CDC) to conduct research on the organism outside the laboratory in accordance with the restrictions outlined in their proposal and the recommendations from ABRAC. Because the research involves not only testing of the recombinant DNA agent, but also involves challenge with the virulent S-2308 derived organism, which is classified as a human pathogen, a CDC waiver

for testing outside the laboratory is considered necessary.

USDA's Animal and Plant Health Inspection Service (APHIS), under 9 CFR Part 103.3 has regulatory jurisdiction regarding approval of the proposed field trial. Assuming a CDC waiver is obtained, Texas A&M then must submit all applicable information to support the request for authorization to conduct the field test to APHIS, including the CDC waiver. APHIS has indicated that by following the procedure discussed above, their review can be expedited. That review will include a site inspection by Veterinary Biologics field office personnel to evaluate containment facilities, access of the test site, disposition of waste materials, etc. The review will also be conducted in accordance with the National Environmental Policy Act (NEPA) and will include an environmental analysis of the proposed field trials. Documentation of the environmental analysis will be made available to the public.

Confinement Working Group In preparation for ABRAC review, OAB arranged for a special ABRAC Confinement Working Group meeting, held in Chicago on November 11, 1988, to take advantage of the expertise at the 41st Annual Brucellosis Research Conference held November 12-13. Dr. John Gorham of the ABRAC chaired the meeting. The purpose of the meeting was to present a common ground of information, to invite recommendations from the research community, and to compare the data presented with the Guidelines being formulated by ABRAC for research on animals outside the laboratory. Pertinent facts about brucellosis and concerns raised at the meeting include the following:

1. Brucellosis is a bacterial disease of cattle that causes abortions. After more than 50 years of eradication efforts, currently costing about $50 million per year, 27 states are free of the disease and only a few southern states (Texas being one) have significant numbers of infected herds.

2. The causative organism, Brucella abortus, is highly infectious for humans causing the disease referred to as Undulant Fever. It is effectively treated with an antibiotic regimen and is not usually fatal.

3. CDC reports that among all human illnesses reported from laboratory research, infections from B. abortus rank highest in occurrence.

4. The vaccine in use today is a living, naturally attenuated strain of Brucella (Strain 19) that is highly immunogenic, but it establishes an infection in vaccinated animals and has several disadvantages as the eradication program progresses. The cattle industry and the USDA have placed a very high priority on the development of an improved vaccine.

5. Ultimately any promising vaccine must be tested in cattle in sufficiently large enough numbers to demonstrate efficacy. All such experiments will involve challenge with the infectious agent. Researchers have questioned whether, as a practical matter, the numbers of animals required can be tested at the NIH/RAC BL-3N containment level.

6. Currently, infected cattle are slaughtered in official USDA establishments; the carcass is subject to inspection and allowed for marketing if the inspection indicates the meat is safe for human consumption.

7. Any experiment outside the laboratory must not only confine the organism to the test site and include controls that protect those engaged in the experiment, but must assure that the test animals are isolated and protected from any accidental exposure to endemic diseases.

USDA DRAFT GUIDELINES FOR RESEARCH OUTSIDE THE LABORATORY INVOLVING BIOTECHNOLOGY

The working strategy for the Guidelines is a follows:

1. The status of the organism will be established based on the existing knowledge of the properties of the unmodified organism, in accordance with principles explained in the Guidelines.

2. The modification of the genetic composition of the organism will be considered based on the knowledge of the properties of that modification.

3. The modification may then raise or lower the status of the modified organism.

4. A table will assign the modified organism to a category (I through IV) which will establish the recommended level of confinement (I through IV).

5. The Principal Investigator will then choose the appropriate methods for the achievement of that level of confinement, subject to review by the IBC or ABRAC, unless exempt. (Currently, no categories are exempt, but future exemptions are anticipated based on experience.)

COMPARISON OF TEXAS A&M PROPOSAL WITH THE DRAFT GUIDELINES

1. Classification of Unmodified Organisms. B. abortus is tentatively classified in the Working Paper as a Status 4 organism. Status 4 organisms are defined in the draft USDA Guideline as:

> "Organisms with potential (a) for serious harm to human health or to managed or natural ecosystems and (b) for transfer of genetic information to other organisms, or for rapid and widespread dissemination in the environment, or for uncontrollable reproduction."

2. Type of modification. The modification to produce the recombinant DNA organism and the characteristics are presented in the Attachment. The live mutant Brucella abortus vaccine is an attenuated organism derived from Brucella abortus strain 2308 as a transposon(Tn5) rough mutant which has never shown reversion to a smooth more virulent form even when passaged through twenty passages of liquid medium or when passaged through Balb/c laboratory mice. Tn5 mutagenesis was performed on Brucella abortus strain 2308 using the method of Smith and Heffren (Infection and Immunity, 55:277-276, 1987) to construct mutants deficient in the gene(s) encoding the enzyme(s) responsible for the synthesis and expression of lipopolysaccharide moiety on the outer membrane of Brucella abortus.

3. Category of Modified Organism/Class of Experiment. ABRAC should consider whether the type of modification raises or lowers the category for the modified organism. However, since the proposed study would also use S-2309 derived B. abortus for the challenge, the class of experiment may be dependent on this unmodified organism.

4. Level of Confinement. If the experiment is a Class IV, Level IV, confinement is required. Level IV confinement requires:

> "The use of good agricultural practices plus all available confinement class principles (i.e., physical, biological, environmental, chemical and scale) appropriate to the organism."

TEXAS A&M PROPOSAL AND CONFINEMENT CONDITIONS

The research planned could involve up to 200 cows divided into 8 treatment groups of 25 pregnant cows each, including a nonvaccinated negative control group and a Strain 19 vaccinated positive control group. One group would be inoculated subcutaneously with 1 x 10^{10} cfu live transposon mutant vaccine. Others groups would be inoculated with killed Brucella abortus

rough cell envelope vaccine derived from the transposon mutant vaccine described above and killed by 1.38 megaRads of Cobalt-60 gamma radiation. After treatment, the unbred cattle would be artificially inseminated from day 60 post-vaccination (PV) until day 120 PV, followed by experimental conjunctival challenge with 1×10^7 cfu Brucella abortus strain 2308 on day 246 PV, following which the cows will either abort or have normal parturitions through day 350 PV.

1. Physical Barriers.

    a. Double fenced facility with one locked and restricted (cattle guard) entrance to prevent accidental release or theft; appropriate biohazard warning signs. All cattle will be continuously maintained in the primary barrier containment area constructed of impervious concrete floors (See diagram in Attachment).

    b. Confinement area monitored at least 4 times daily.

    c. All animals tattooed for identification.

    d. All solid and liquid wastes collected in retention pond, held 60 days, and cultured for Brucella before release.

    e. Abortions collected, double contained for transport, and incinerated.

    f. Samples transported in sealed durable containers inside durable rigid containers.

    g. Contaminated materials placed in leak-proof durable containers for autoclaving or transport to incinerator.

    h. Animals inoculated with live rDNA or otherwise derived products to be euthanized for necropsy and cremation.

    i. At least 12 sentinel cows will be placed adjacent to, and down-stream from, the facility and monitored.

2. Biological Barriers

    a. The mutant strain has shown no shedding in studies conducted in mice and goats.

    b. Organism survives but does not replicate outside narrow host range.

3. Environmental Barriers

a. Site location.

b. Organisms in air and water will dilute rapidly to below infective dose. Use of aerosols in facility will be minimized.

c. Attachment provides documented survival information. Environmental conditions re: retention pond should provide for organism destruction within 60 days.

d. Protective clothing and other contaminated materials not incinerated will be autoclaved.

e. Personnel will remove protective clothing and shower before leaving the facility, and wash immediately after handling animals and materials.

4. Chemical Barriers

a. 4% formalin solution will be used to decontaminate abortion sites, and outside packaging for transportation of contaminated materials.

b. At the termination of the experiment, the facility will be washed down with low pressure water and disinfected with dilute chlorox solution and left vacant for thirty days prior to initiating any other experiment.

5. Scale

Up to 200 animals will be used.

6. Personnel Safety Measures

a. All personnel will be at least 18 years of age, and fully instructed and examined regarding the biosafety procedures and hazards.

b. Protective clothing will be worn: lab coats, overalls, smocks, and boots in the work area; gloves, goggles and molded surgical masks when handling animals or contaminated materials.

c. Workers will shower before leaving the facility and wash their hands after any contact with animals or materials involving the organisms.

d. Eating, smoking, drinking, mouth pipetting and cosmetics application will not be permitted in work areas.

e. Serum samples will be collected for serological surveillance at the start of employment and quarterly thereafter.

f. Use of hypodermic needles with leur-lock syringes and sealed septum vials will be limited to only when absolutely required, e.g., inoculation and sample collection. All sharp objects will be placed in puncture-resistant containers before autoclaving.

BIOSAFETY EVALUATION FRAMEWORK

The following framework is suggested to assist ABRAC in a systematic review and discussion of the biosafety aspects of the proposal.

A. Personnel Safety

Sources of potential exposure:

a. inoculation
b. collection of samples
c. removal of abortions
d. handling infective animals
e. waste products
f. air fomites transmission

Are the safety measures proposed adequate to protect employees on site? Are the provisions of medical surveillance of employees adequate to prevent secondary transmission? If not, what additional precautions are needed and why?

B. Potential Vectors for Transmission Outside the Testing Site

1. Employees

Are the provisions adequate to prevent workers from transmitting the organism? If not, what additional precautions are needed and why?

2. Test animals

Are the physical confinement structures, procedures and security measures adequate to assure isolation during the test period? If not, what additional measures are needed and why?

Are the procedures for disposal of animals given live organisms after the experiment is terminated adequate to prevent transmission? If not, what additional measures are needed and why?

3. Animal wastes

Are the procedures for collecting, storing, testing and release of animal waste adequate? If not, what additional procedures are needed and why?

4. Other contaminated materials from the test site

Are the procedures for handling, transporting, autoclaving or incinerating contaminated materials, and the proposed use of chemicals for decontamination adequate to prevent transmission? If not, what additional measures are needed and why?

5. Aerial transmission

Considering the pool size of organisms that will be used and atmospheric dilution, can aerial transmission be discarded as an environmental concern? If not, why not?

6. Other potential vectors

Are there other potential vectors (e.g., insects, birds, rodents, or other wildlife) in the vicinity? What is the basis for concern or lack of concern? What, if any additional precautions are needed to mitigate the effect of any other potential vectors of transmission identified?

7. Other issues

Do any additional issues need to be addressed in arriving at an ABRAC recommendation?

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November 15, 1988

Recommended Guidelines for Evaluation of Brucellosis Vaccines (Live or Inactivated) Developed through rDNA or other Methods with Testing of Efficacy by Virulent Challenge Under an Optimized Combination of BL1N, BL2N, BL3N requirements.

1 Containment area for animals will be double fenced with one locked and restricted entrance (cattle guard) to avoid accidental release or theft.

2. Containment area for animals will be monitored for the integrity of the security of the facility at least four times daily.

3. All liquid and solid wastes from the concrete area will be collected by low pressure washing with water into the retention pond, which is no greater than 3 ft deep with a large surface area, held for 60 days, and cultured for *Brucella* prior to release. The retention pond is fenced with steel mesh fencing with two barb wires above the mesh fencing to restrict entry of any domestic animals, except for the sentinel herd. According to published data (Respess, R. O. et al. Seasonal Variations in Selected Physiochemical Conditions of a Small Lake in Brazos County, Texas. Southwest naturalist, 17:249-263, 1972) the mean temperature of the water 6" beneath the surface will be no less than 8° C and have a slightly alkaline pH resulting in an estimated 60 day survival of *Brucella abortus* in the retention pond. At the end of the 60 day holding period, water samples will be collected, concentrated, and bacteriologically cultured for the presence of *Brucella abortus* on semi restrictive and restrictive media in the presence of carbon dioxide at 37.5°C for 7 days.

4. The animal containment area will comply with Federal Law and animal care requirements.

5. Biohazard warning signs and special requirements for entry will be posted at all doors or gates to animal work area.

6. The Biohazard warning sign will list the name and address of laboratory director, infectious agents and animals involved.

7. The cattle will be maintained in animal containment areas which will be double fenced, i.e. primary and secondary barriers, constructed of steel tubing with impervious concrete floors, sheet steel ventilated sheds for ease of cleaning and disinfection. The secondary fencing is 66" high and constructed of steel mesh and wooden post fencing on top of which are two strains of barb wire.

8. Minimization of aerosols in the contaminated areas will be employed at all times.

9. Animals of any species not involved in the experiment will be disallowed in contaminated work areas.

10. Facilities, including laboratories and animal containments, will be subject to unannounced local, state and federal inspection prior and during experimentation.

Attachment

11. In the case of abortion induced by *B. abortus*, the placenta and/or fetus will be carefully double contained and the site disinfected with 4% formalin. The fetal and placental specimens, doubly contained in plastic bags, will be transported in sealed durable containers inside sealed durable ridged containers which are disinfected externally prior to being transported to necropsy or isolation facilities.

12. No less than twelve naive, non-immune susceptible reproducing sentinel cows will be stationed immediately adjacent (within one foot) and down-stream to the double-fenced animal containment areas; however, other than these sentinels, no other cattle will have adjacent access. Blood samples will be collected and serologically tested for antibodies to *B. abortus* from sentinel cattle at three month intervals throughout the period of experimentation and semiannually thereafter for one year or one reproductive cycle following the termination of the experiment.

13. Permanently mark (tattoo) all animals.

14. At the completion of the experiment, cattle inoculated with *killed* products will be either: (a) euthanatized for necropsy and cremated, or (b) slaughtered at establishments subject to Federal Meat Inspection upon approval by the USDA/FSIS.

15. At the completion of the experiment, cattle inoculated with *live* rDNA or otherwise derived products will be euthanatized for necropsy and cremation.

16. In regard to live rDNA derived *Brucella abortus* or otherwise developed vaccines, these vaccines will be evaluated in mice and/or small ruminants under BL3N conditions for fate of organism, shedding, pathogenicity, and/or efficacy before evaluations in cattle in outdoor controlled containment will begin.

17. Persons less than 18 years will not be employed.

18. Persons in containment work area will wash their hands after any contact with animals or materials involving organisms.

19. Eating, smoking, drinking, mouth pipetting and cosmetics application will not be permitted in work areas.

20. Policies, protocols and procedures for handling contaminated animals and materials will be published and presented to all laboratory workers who will be subsequently given a written examination to insure their comprehension of the safety procedures.

21. Protective clothing - lab coats, overalls, smocks will be worn in laboratory and animal work areas. Protective clothing in the animal work areas will be changed upon every entry and exit from the facility. Laboratory clothing will be autoclaved before laundering.

22. Skin and mucous membranes will be protected against exposure to the agent by wearing protective gloves, goggles and molded surgical masks when handling animals or contaminated materials.

23. Any accidental exposure will be reported in writing immediately to the lab director for medical consultation and treatment where required.

24. All laboratory personnel will have their serum samples collected at the initiation of employment and quarterly thereafter for serologic surveillance. Serum samples will be maintained as a historical human serum bank.

25. Personnel will shower when leaving the animal containment facility.

26. In order to avoid self-inoculation, use of hypodermic needles only with luer-lock syringes and sealed septum vials will be limited to only when absolutely required, e.g. inoculation, sample collection. All sharp objects will be placed in puncture-resistant containers before autoclaving.

27. Samples collected in the animal containment areas will be transported in sealed durable containers inside sealed durable rigid containers which are disinfected externally.

28. Contaminated materials will be placed in leakproof durable containers for autoclaving or transport to incineration.

29. All experimental protocols must have approval of the following local committees before initiation: IBC, Committee on Infectious Biohazards, University Laboratory Animal Care Committee, Laboratory Animal Resource and Research Committee.

30. A diagrammatic illustration of the design of the facility is provided in the attached document (Exhibit 1).

31. The details of the area surrounding the facility are given in the enclosed map (Exhibit 2).

32. In the event of death or necessity for euthanasia and necropsy, carcasses will be removed in plastic lined buckets of front end loader equipment directly to the necropsy laboratory, while live animals for euthanasia will be transported by livestock trailer to the necropsy facility one mile from the control containment facility following which all equipment will be disinfected with dilute chlorox (sodium hypochlorite) solution.

33. At the termination of the experiment, the facility will be washed down with low pressure water and disinfected with dilute chlorox solution and left vacant for 30 days prior to initiating any other experiment.

Comments and Rationale

General:

1. Guidelines and regulations should be written to *facilitate* research and development while protecting the environment.

2. Guidelines for developing and testing biologics should be clearly written to avoid confusion in obtaining approval.

3. Requirements for approval to evaluate biologics should be clearly stated to avoid and eliminate overlapping jurisdiction at local, state and federal levels, i.e., IBC, NIH, USDA/APHIS/Veterinary Biologics, USDA/OAB/ABRAC.

4. Delegate the authority having jurisdiction first to the local IBC, then to *either* USDA/OAB/ABRAC *or* USDA/APHIS/Vet Biologics or NIH but *not* all three.

5. Approaches to improved biologics has changed significantly, but the products are *not* significantly different from those produced by conventional methods, in fact virtually all new biologics are better characterized and safer than the products they replace.

6. Questions pertaining to new biologics should be focused on the safety, efficacy and intended use not on the methods employed in their development or production.

7. The approach to meet the containment needs should be pragmatic because of the high costs and time involved.

Relevant Data:

1. In the case of mammalian brucellosis, there is no evidence of arthropod transmission. Although *Brucella* have been recovered from mosquitoes, flies, fleas, and ticks under experimental conditions, the transmission of brucellosis from one infected herd to a clean herd by these insects have not been shown. (Margaret E. Meyer. Epidemiological Odds and Ends. In: Bovine Brucellosis-An International Symposium. College Station, TX. Crawford, RP and Hidalgo, RJ, eds. Texas A&M University Press, 1977, p. 135-142.

2. In the case of bovine brucellosis, the pregnant *Brucella abortus* infected cow is the *only* major factor in disease transmission to other cattle and man.

3. *Brucella abortus* has been documented to only survive, *not* replicate, outside a narrow host range. Although *Brucella abortus* has been isolated from sheep, horses, swine, birds, dogs, and other non-domestic or wildlife species, there is little doubt that in the USA *Brucella abortus* has its primary reservoir in cattle. Various avian species are susceptible to and experimental studies suggest they are rarely infected for any length of time. (Chapter 8. Epidemiology of Bovine Brucellosis. In: Brucellosis Research: An Evaluation. A Report of the Subcommittee on Brucellosis Research. Committee on Animal Health. Washington, DC: National Academy of Sciences, 1977, p. 139-158.

4. The ID_{50} of *B. abortus* for cattle has been estimated in publications to be $\approx 1 \times 10^4$ cfu.

5. Man has been demonstrated to have the following decreasing susceptibility to *Brucella spp*: *B. melitensis* > *B. suis* > *B. abortus* and man is less susceptible to *B. abortus* than cattle are.

6. Some published *B. abortus* environmental survival studies: See enclosed Table 14-4.

7. Currently under Federal law, cattle infected with field strains of *Brucella abortus* are sold in markets and slaughtered in USDA/FSIS sanctioned abattoirs.

8. Human brucellosis caused by *Brucella abortus* can be chemotherapeutically treated effectively with antibiotic regimen.

9. NIH classifies all Brucellae as Class 3 including *Brucella abortus* S19 which is a USDA/APHIS/VS licensed and widely disseminated vaccine, i.e. under this classification system, all S19 vaccine would have to be produced under BL3N confinement.

10. In over 2360 cattle, bison, goats, sheep and/or elk experimentally challenged with prototype virulent field strain *Brucella abortus* S2308, no confirmed evidence of transmission to any other domestic livestock species has occurred from 1971 through 1988.

In Case of Failure:

1. If a barrier fails, what are the acceptable limits of failure? Answer: Seroconversion but no infection of sentinels.

2. How will failure be measured? Answer: Seroconversion, clinical signs and bacteriologic culture of sentinels.

3. What procedures will be used to correct emergency situations? Answer: Seroconversion test, euthanasia and cremation.

4. What is the weakest link in the containment? Answer: Accidental release of exposed pregnant cows; Prevention - Secure containment to prevent escape or release.

5. What is the cost of maintaining barriers versus cost of correction in case of failure? Serologic surveillance and slaughter in restricted zones may well be more cost effective and efficient than maintaining barriers, but *both* are *essential*.

Proposed Containment:

1. It is *not* logical to prevent the evaluation of new killed or live *Brucella abortus* vaccines by virulent challenge in endemic zones where not only *containment*, but *isolation* to prevent accidental entry of field strain *Brucella abortus* is of equal importance.

2. With the enclosed proposed controlled containment management system, the effects of dilution in air or water plus the effects of temperature, pH, and sunlight significantly reduce the potential for transmission below $\approx 1 \times 10^4$ cfu ID_{50} threshold for cattle, i.e. *Brucella spp* have rarely been documented to be transmitted by inert objects.

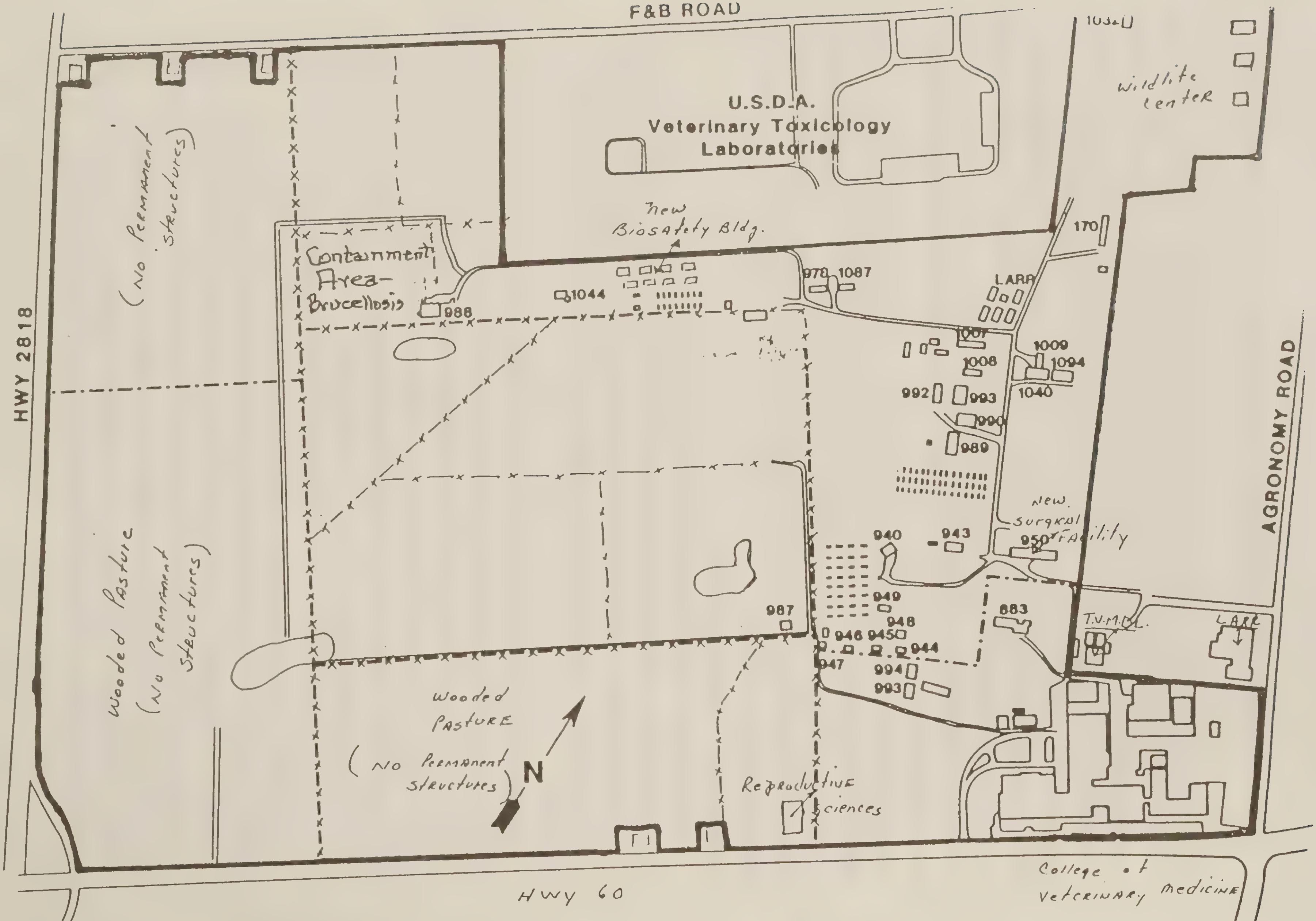
3. We propose that the enclosed controlled containment management system will provide more than an adequate envelope or barrier around *Brucella abortus* live rDNA or killed vaccination/challenge experiments to prevent accidental exposure to man, animals, or environment, because we have:

   (a) Established management protocols
   (b) Isolated *Brucella abortus* at the source
   (c) Reduced and minimized risk of exposure
   (d) Increased distance from other cattle through dilution and space
   (e) Built in redundancy through primary and secondary barriers with sentinels to monitor accidental exposure
   (f) Documented experimental animals and treatments

(g) Documented monitoring and surveillance protocols for laboratory workers in case of accidental exposure
(h) Identified issues related to facility location and environment.
(i) Strategized the assessment of live rDNA vaccines in rodents and/or small ruminants under BL3N conditions for fate of organism, pathogenicity and shedding before evaluation in cattle in outdoor controlled containment areas would begin and therefore,
these protocols, properly employed, will specifically prevent any implied or real negligence, which is the failure to excise due care for man, cattle and the environment.

DETAILS OF THE PROPOSED EXPERIMENTS

1. A series of experiments are planned in cattle for evaluating killed and live *Brucella* vaccines. Each of these experiments will contain at least three treatment groups of 25 pregnant cows each - Group 1 - nonvaccinated negative controls, Group 2 - strain 19 postive controls and other experimental treatment groups to include at least two groups, killed cell envelope preparations from rough *Brucella* mutants and live transposon deletion mutants of *Brucella abortus*. It is possible that the proposed facility could contain a total of eight treatment groups of 25 pregnant cows each including the nonvaccinated controls and strain 19 control vaccinates.
2. The cattle will be continuously maintained in the primary barrier containment area constructed of impervious concrete floors.
3. The unbred experimental cattle will receive the treatments as outlined above on day 0 by subcutaneous parenteral injection, followed by artificial insemination from day 60 post-vaccination (PV) until day 120 PV, followed by experimental conjunctival challenge with 1×10^7 cfu *Brucella abortus* strain 2308 on day 245 PV, following which the cows will either abort or have normal parturitions through day 350 PV.
4. With regard to the products to be tested:
    a. Killed *Brucella abortus* rough cell envelope vaccine - the organismal source of these cell envelopes was derived from *Brucella abortus* strain 2308 via transposon mutagenesis using Tn 5 to produce rough mutants which are subsequently killed by 1.38 megaRads of ^{60}Co gamma radiation and processed for cell envelopes and combined with a USDA approved adjuvant.
    b. Live mutant *Brucella abortus* vaccine-this attenuated organism was derived from *Brucella abortus* strain 2308 as a transposon (Tn 5 rough) mutant which has never shown reversion to a smooth more virulent form even when passaged through twenty passages on liquid medium or when passaged through Balb/c laboratory mice. A dosage comparable to the recommended adult dose for the USDA Strain 19 will be injected subcutaneously into the experimental cattle. Tn 5 mutagenesis was performed on *Brucella abortus* strain 2308 using the method of Smith and Heffren (Infection and Immunity, 55:277-276, 1987) to construct mutants deficient in the gene(s) encoding the enzyme(s) responsible for the synthesis and expression of lipopolysaccharide moiety on the outermembrane of *Brucella abortus*. Subcutaneous inoculation of this organism into goats with subsequent weekly sample collection from: tears, saliva, urine, feces, and blood failed to demonstrate the organism in any of these specimens: therefore, no shedding occurred. No organisms were isolated from 50 different tissues collected from the goats at 150 days post inoculation, nor was there any evidence of lesions or pathogeniecity induced in the goats by this transposon mutant of *Brucella abortus*.

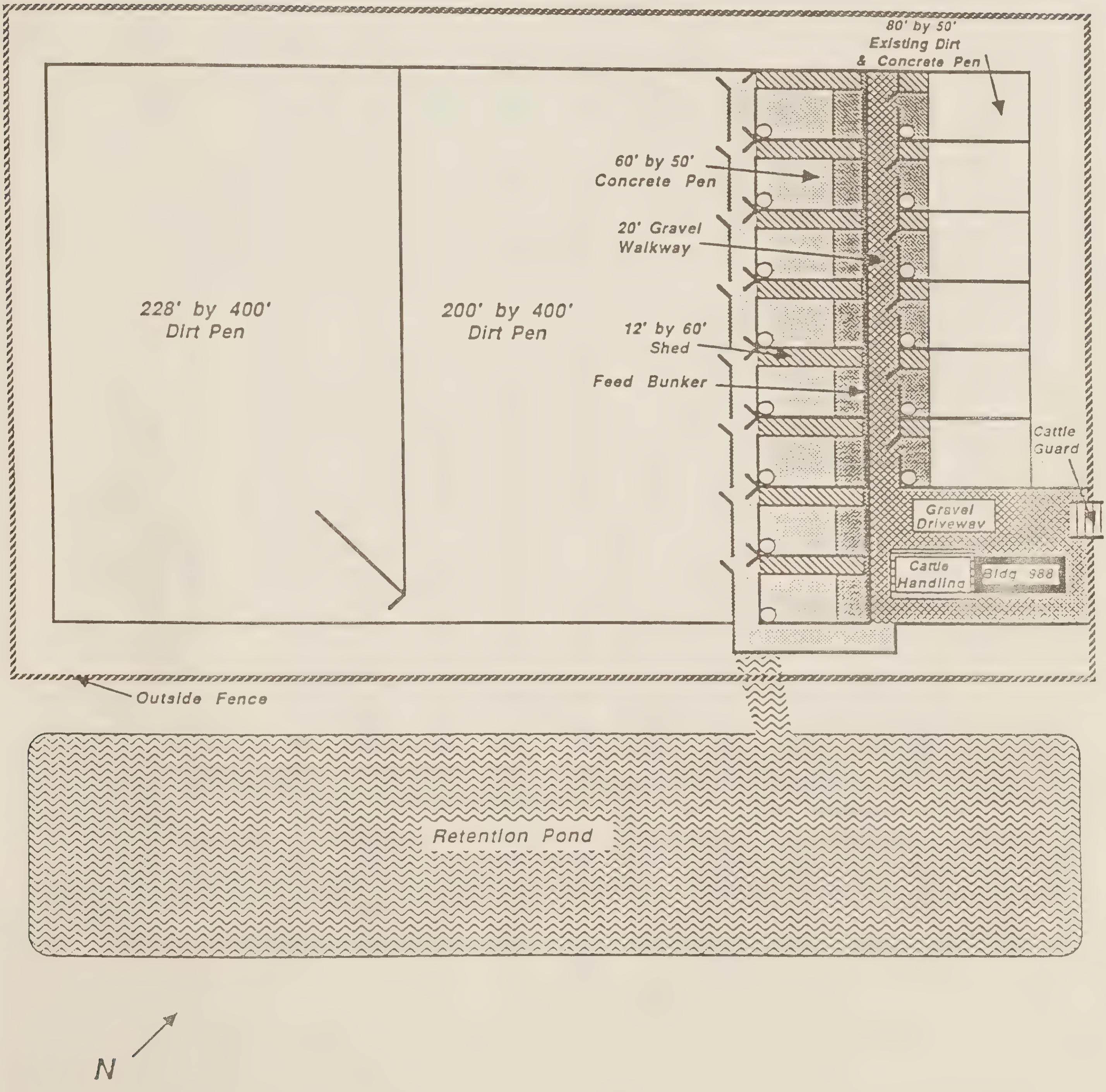


Attachment 5. Veterinary Medical Park

Exhibit 2

—x—x Fences

Brucellosis Cattle Facilities



Survival of *Brucella* in Sterilized
and Untreated Bovine Manure and Soil
at Various Temperatures

Temp. (C)	Bovine Manure		Soil	
	Sterilized	Un-treated	Sterilized	Un-treated
37	188 Days	0 Days	100 Days	5 Days
25	286 Days	29 Days	156 Days	29 Days
8	227 Days	385 Days	93 Days	188 Days
-3	325 Days	121 Days	52 Days	188 Days
-40	670 Days	670 Days	670 Days	670 Days

Kuzdas, C. E. and Morse, E. V. Cornell Vet..
44:216-228 (1954).

Table 14.4. Survival times of *B. abortus* under various environmental conditions

Medium	Temperature or season	Environmental conditions	Survival time (days)	Reference
Uterine exudate	February	Placed on ground	10	Cotton. Jour. Am. Vet. Med. Assoc., 1919, *55*, 504.
Placenta, fetal organs	Winter and spring	Covered with leaves in forest	135	Cotton. Jour. Am. Vet. Med. Assoc., 1919, *55*, 504.
Milk	15 C	Milk samples from infected cows	38	Huddelson, Hasley, and Torrey. Jour. Inf. Dis., 1927, *40*, 352.
Butter			142	Carpenter and Boak. Am. Jour. Pub. Health, 1928, *18*, 743.
Cheese	4.4 C		180	Gilman, Dahlberg, and Marquardt. Jour. Dairy Sci., 1946, *29*, 71.
Grass	10-70 F, February	0.6 in. rain	6	Ky. Agr. Exp. Sta. Bull. 43, 1931, p. 14.
	60-81 F, May	Sunny	<1	Ky. Agr. Exp. Sta. Bull. 43, 1931, p. 14.
	36-70 F, November		5	Ky. Agr. Exp. Sta. Bull. 43, 1931, p. 14.
Open plate cultures	October and November	Sunny	2-3	Ky. Agr. Exp. Sta. Bull. 43, 1931, p. 14.
Water	−40 C		800	Kuzdas and Morse. Cornell Vet., 1954, *44*, 216.
	37 C, 25 C, 8 C		57	
Infected guinea pig carcass	January (Wisconsin)	Placed on ground	44	Kuzdas and Morse. Cornell Vet., 1954, *44*, 216.
	June and August (Wisconsin)	Placed on ground	1	Kuzdas and Morse. Cornell Vet., 1954, *44*, 216.
	January (Wisconsin)	Buried	29	Kuzdas and Morse. Cornell Vet., 1954, *44*, 216.
Meat and salted meat	0-20 C		65	Prost. Ann. Univ. Marie Curie—Sklodowska, Lublin, Poland, 1957, *12*, 163.
Manure pit	158 F	In tubes at bottom of pit	<4 hours	King. Jour. Am. Vet. Med. Assoc., 1957, *131*, 349.
Manure pit		In tubes at top of pit	2	King. Jour. Am. Vet. Med. Assoc., 1957, *131*, 349.
In manure	12 C		250	Plommet. Anns. Recher. Vet., 1972, *3*, 621.

RECOMMENDATION OF THE USDA AGRICULTURAL BIOTECHNOLOGY RESEARCH ADVISORY COMMITTEE (ABRAC) ON THE TEXAS A&M BRUCELLOSIS BIOSAFETY RESEARCH PROPOSAL

January 6, 1989

It is recommended that before any field experimentation is conducted in cattle there must be assurance that the transposon Tn5 rough mutant Brucella strain is tetracycline sensitive.

The biosafety and confinement conditions for the Brucella research proposed by Texas A&M will be adequate to ensure no unreasonable risk to human safety and the environment if the additions and clarifications listed below are added to the protocol. With these additions and clarifications, the biosafety provisions will provide a level of protection greater than that which occurs naturally in an infected area. The recommended additions and clarifications for biosafety include:

a. Full face mask respirators should be used during challenge and when handling highly infective tissues.

b. A shower facility should be provided at the experimental site and procedures for disinfecting boots should be provided.

c. A comprehensive medical surveillance program should be developed and instituted at the expense of the Institution to include, in addition to those elements listed in the protocol:

(i) Appropriate procedures for informing employees of potential risks and obtaining informed consent to participate in the study,

(ii) Serologic surveillance with appropriate antigens to detect antibodies against both the mutant strain and the challenge strain to be continued for an appropriate time after completion of the experiment, and

(iii) In case of accidental exposure, a treatment protocol should be available.

d. Pulsating, high-voltage electric fencing (similar to the Australian anti-dingo system) for the animal facility and lagoon should be adequate to exclude animals, e.g., raccoons, coyotes, dogs, cats, and deer.

e. Full time electronic theft-deterrent surveillance

systems for the test site should be provided.

f. A rodent and insect control program should be provided.

g. The wastes in the lagoon would be held for at least 60 days following termination of the trial and cleanup and cultured for both the mutant vaccine and challenge strains before release.

h. The scheduling of the study should take advantage of environmental conditions detrimental to dissemination and survival of the organisms into the environment.

i. Sentinel animals should include a) a group bred at the same time as the experimental animals to assure susceptibility and response to infection (i.e., abortion) and b) a group of nonpregnant animals. Sentinels should be quarantined for an appropriate period of time before use. Serological testing should be adequate for both the mutant vaccine and challenge strains.

j. If a sentinel animal seroconverts or shows clinical signs suggestive of brucellosis, the animal should be sacrificed and cultured immediately. If the culture is positive for Brucella, the IBC and APHIS should be notified and appropriate actions, e.g. increased surveillance, taken.

k. Good agricultural practices will be followed.

HOLLINSHEAD DRAFT 1/6/89

Purpose: The USDA guidelines are designed to specify practices and procedures for agricultural research outside the laboratory involving biotechnology. The guidelines establish fundamental principles upon which the safety of specific experiments and organisms can be evaluated and upon which confinement practices can be based.

The Guidelines apply to all research funded by USDA. If experiments are submitted for review to another Federal agency, OAB may be consulted by agency or submitter for rapid determination that the review is in compliance with USDA ~~ABRA~~C guidelines. Groups or individuals not funded by Federal sources are encouraged to comply with ABRAC guidelines.

The~~se~~ guidelines do not preclude compliance with regulatory requirements of Federal, state or local agencies.

Hollinshead handout

IV-A-4. All experiments with Status 5 organisms (II-A-5) or which involve genetic modifications of Category Type 2 (II-B-3-b(3)) or Type 3 (II-B-3-c) should have IBC and ABRAC review and USDA approval.

V. Roles and Responsibilities.

V-A. Institution. Each Institution conducting or sponsoring agricultural research outside the laboratory involving biotechnology is responsible for promoting safe research. [It is recommended that the Institution] Fulfilling this responsibility requires at least the following activities:

V-A-1. Establish and implement policies which provide for the safe conduct of research in compliance with the Guidelines;

V-A-2. Establish or associate with an Institutional Biosafety Committee (IBC) that meets the requirements set forth in Section V-B-1 and carries out the functions detailed in Sections V-B-2 and 3;

V-A-3. Require that principal investigators (PIs) responsible for agricultural research outside the laboratory involving biotechnology comply with the Guidelines and assist them in doing so;

V-A-4. Identify its IBC members by name, area of expertise, and affiliation in a report to the Office of Agricultural Biotechnology (OAB) (1) when the IBC is formed, [(2) after initiation of these Guidelines,] and (3) as IBC membership changes;

[V-A-a. Forward to OAB all comments submitted by the public to the institution on IBC actions.) [WE RECOMMEND MAKING THIS A REQUIREMENT OF THE IBC INSTEAD OF THE INSTITUTION; ALSO, EXPANDING TO INCUDE OTHER RECORD-KEEPING REQUIREMENTS--AS DETAILED LATER]

V-A-5. Make available to the public all information on

accepted with sense of ABRAC changes 1-6-89

experiments conducted at the institution involving biotechnology, unless it contains confidential business information, (as defined in the definitions section, or unless its disclosure is prohibited by state or Federal law; furthermore, notify the public, whenever applicable, that appeal procedures exist for challenging the withholding of CBI and/or for obtaining a general description of the material withheld (as explained in section V-D).

V-A-7. Assure that the IBC reports promptly to OAB any significant problems with implementation of these Guidelines; and

V-A-8. Assure that the IBC reports to OAB, within [15] 3 days of occurrence, any research-related accidents that have resulted or could result in human disease or illness or unintentional release to the environment of organisms being studied. [any research-related accidents or diseases involving workers... animals, or plants.]

V-B. Institutional Biosafety Committee.

V-B-1. Membership and procedures. The IBC should:

V-B-1-a. Include at least six members so selected that, as a group, they have experience and expertise in [recombinant DNA, recombinant RNA, and other agriculture-related technologies] agricultural-related biotechnology; and the capability to assess safety of agricultural research outside the laboratory; [with reference to human health and managed and natural ecosystems.] Technical experts shall collectively have expertise in ecology, biology, biological safety, risk

assessment, and physical and biological containment of organisms.

V-B-1-b. Include at least two members not affiliated with the Institution or with other institutions conducting agricultural research and development, and representing the interests of the community, such as, (i) members of state and local public health, environmental, or agricultural agencies, {LANGUAGE COMBINED HERE TO SHORTEN} (ii) persons active in human or animal health concerns, or (iii) persons active in agricultural and environmental concerns. (Employment of an IBC member solely for purposes of membership on the IBC does not itself make the member an institutionally affiliated member.)

IBCs are strongly encouraged to use consultants and adjunct personnel with expertise in institutional policy issues, applicable laws and regulations, standards of professional conduct and practice, community attitudes and the environment, private sector/industry issues, and particular research issues, as needed. An existing IBC may be expanded if necessary to provide the expertise required for the USDA Guidelines.

[Existing IBCs may be expanded, as necessary, to provide the expertise recommended by these Guidelines.]

V-B-2. Review and meetings. In order to comply with these guidelines:

V-B-2-a. IBCs shall notify the public of all upcoming meetings and decisions regarding research proposals, in a major local newspaper at least 10 working days prior to the meeting or decision;

V-B-2-b. IBC shall open meetings to the public

whenever possible, including the opportunity for the public to make comments at the meetings, consistent with protection of privacy and proprietary interests and

V-B-2-c. No member of an IBC may participate in the review or approval of a project in which he or she has been or expects to be engaged, or in which he or she has or expects to have [a direct] any financial interest, except to provide information requested by the IBC. [IBC members who are not or have not been engaged in a project should not participate in the review or approval of that project if they have a direct financial interest in it.]

V-B-3. Functions. [It is recommended that the IBC:] In order to comply with these Guidelines, IBCs shall:

V-B-3-a. Review research projects being conducted, or accepted for review, by the Institution for compliance with the Guidelines and approve those projects that it finds are in conformity with the Guidelines;

V-B-3-b. Report promptly to OAB any significant problems with implementation of these Guidelines;

V-B-3-c. Report to OAB within [15] 3 days of occurrence any research-related accidents that have or could result in human disease or illness, or unintentional release to the environment of the organisms being studied. [any research related accidents or disease involving workers, and V-B-3-c-(2). any research-related accidents or disase involving animals or plants.]

V-B-3-d. Maintain complete documentation of information reviewed for each experiment for 5 years from the

date of commencement of the research, including copies of all data, conclusions, and reports pertaining to the experiment that were reviewed by the IBC.

V-C. Principal Investigator. On behalf of the Institution, the PI is responsible for conducting biotechnological research in a safe manner. As part of this responsibility, the PI [should] shall:

V-C-1. Determine whether experiments are covered by the Guidelines and follow the recommended procedures of the Guidelines;

V-C-2. Instruct and train project staff in practices and techniques to [insure] maximize safety in procedures for dealing with accidents;

V-C-3. Report promptly to OAB or the IBC any significant problems with implementation of these Guidelines; and

V-C-4. Report to the IBC as soon as it is recognized, so the IBC can report to OAB within [15] 3 days any research-related accidents that have or may cause illness or disease in humans, or that have or may involve unintentional release to the environment of organisms being studied. [any research-related accidents, illnesses, or disases involving workers... plants, or animals.]

V-D. United States Department of Agriculture (USDA).

{RECOMMEND LEAVING AS IS, EXCEPT ADDING THE FOLLOWING AT THE END OF THE SECTION:}

V-D-1. Assistant Secretary for Science and Education. The Assistant Secretary for Science and Education is responsible

for the development and implementation by USDA of all matters and functions pertaining to agricultural research conducted or funded by the Department involving biotechnology. This includes the development and implementation of guidelines for research activities. The Assistant Secretary also acts as liaison on all matters and functions pertaining to agricultural research in biotechnology between agencies within the Department and between the Department and other governmental, educational, and private organizations and individuals.

The Assistant Secretary oversees the implementation and utilization of these Guidelines.

V-D-2. Agricultural Biotechnology Research Advisory Committee (ABRAC). The ABRAC makes recommendations to the Assistant Secretary on scientific and technical matters concerning agricultural research outside the laboratory involving biotechnology. ABRAC is responsible for:

V-D-2-a. Recommending additions and alterations to research guidelines and protocols and

V-D-2-b. Providing advice to other Federal and state agencies and IBCs on agriculture-related biotechnological research projects.

V-D-3. Office of Agricultural Biotechnology. The Office of Agricultural Biotechnology has the responsibility to provide administrative support for developing and coordinating departmental policies and activities pertaining to biotechnological research and to perform related interagency and public liaison functions. Inquiries to USDA concerning biotechnology can be directed to OAB. The OAB is specifically

responsible for the following:

V-D-3-a. Announcing ABRAC meetings and agendas;

V-D-3-b. Providing the ABRAC executive secretary;

V-D-3-c. Assisting in the development and implementation of policies and procedures pertaining to the conduct of research and experimentation in biotechnology; and

V-D-3-d. Maintaining records of research and regulatory activities carried out under the biotechnology authorities of the Secretary of Agriculture.

<u>V-D-3-e. Referring inquiries about regulatory reviews to other federal agencies and/or offices within USDA, as appropriate.</u>

<u>V-D-3-f. Establishing procedures for IBCs to follow regarding appeal and review of confidentiality claims;</u>

<u>V-D-3-g. Establishing procedures to notify the public of the general nature of withheld information where that information has been requested.</u>

January 6, 1989

USDA GUIDELINES FOR RESEARCH

OUTSIDE THE LABORATORY

INVOLVING BIOTECHNOLOGY

III. Confinement principles.

This Section outlines principles and practices of confinement for use in conducting and handling biotechnological research performed outside the laboratory and for appropriate selection of mechanisms of confinement or to define those available ~~thereto~~. The primary goal is to limit the potential of organisms or their products for transfer of genetic information to other organisms, for rapid and widespread dissemination from experimental areas, or uncontrollable reproduction in cases where transfer, dissemination, or reproduction presents the potential for unreasonably adverse effects on human health or on managed or natural ecosystems.

III-A. Application of confinement principles. It is the responsibility of the principal investigator to select and propose the appropriate confinement level for each study. In general, a higher level of confinement is required for organisms included in a higher status than for organisms included in a lower status. A greater degree of confinement can be achieved by increasing the stringency of the confinement practices or the number and variety of confinement practices.

Degrees of confinement vary from simple [isolation, border rows, or fencing] to elaborate barriers and monitoring for inadvertent escape. Likewise, as indicated by Section II, the

properties of organisms and their products differ with respect to their potential for transfer of genetic information to other species, rapid and widespread dissemination, and uncontrollable reproduction and their potential for unreasonably adverse effects on human health or on managed or natural ecosystems. The properties and characteristics of sites or locations selected for field tests will also vary. The level of confinement needed to limit the transfer of genetic information, dissemination, or reproduction of an organism or its products from experimental area must correspond to the properties of the organism, the characteristics of the experimental area, and the design of the experiment. For experiments using multiple organisms, the level of confinement should be that required by the organism of the highest status (i.e., most pathogenic).

Some organisms, by virtue of their exotic nature or ability to cause grave harm, must not be tested outside the laboratory, regardless of the level of confinement. Other organisms, because of their low harm or self-limiting nature, may require no or minimal confinement.

III-B. Confinement Classes. Confinement for organisms and their products, whether plant, animal, or microbe, modified using biotechnology and to be used for experimentation outside the laboratory are grouped into five classes. Illustrative examples are given for each class of confinement, but these are not inclusive of all options available to experimenters. Furthermore, within each class of confinement, an increased degree of stringency can be designed to provided for each specific organism and experimental area.

III-B-1. Physical. Physical barriers can be used to limit the survival and dissemination of organisms or their products outside the experimental area. Physical barriers include border rows, geographical isolation, dams, soil terraces, tillage, fences, screens and meshes, and impervious or plastic barriers.

III-B-2. Biological. Biological barriers can be used to limit survival and dissemination of organisms or their products outside the experimental area and to limit the transfer of genetic information from the test organism to other organisms. Biological barriers include genetic modifications that disable the organism, that produce sterility, and that reduce the ability of the organism to survive or to escape predators. Removal of reproductive organs and removal of organisms that are hosts for the test organism can be used to aid confinement. Natural biological decay, e. g., normal death, is a further barrier.

III-B-3. Environmental. Environmental conditions can be used to limit reproduction of the organism and, in some cases, to limit survival or dissemination of organisms or their products outside the experimental area. Environmental variables, which are reproduction-limiting, include temperature, water supply, humidity,and photoperiod.

III-B-4. Chemical. Chemicals can be used to limit survival and reproduction of organisms and or their products outside the experimental area and to limit transfer of genetic information from the test organism to other organisms. Chemical treatments include herbicides, fungicides, insecticides, disinfectants and other materials toxic to the test

organism, pH alterations, gametocides and other chemicals acting as reproductive control agents, elimination of essential nutrients.

III-B-5. Scale. By decreasing the number of organisms used in an experiment or decreasing the land area, the possibility of rapid and widespread dissemination may be reduced.

III-C. Confinement levels.

Four levels of confinement, ranging from good agricultural practices to strict measures to limit transfer of genetic information to other organisms, rapid and widespread dissemination, or uncontrollable reproduction of test organisms and their products, are described below. There is a continuous progression of confinement from good agricultural practices (Confinement Level 1) to maximum confinement (Confinement Level 4). Each Confinement Level Is based upon good agricultural practices plus additional confinement methods to achieve progressively greater confinement. This progression is based on the level of protection provided for human health or managed or natural ecosystems. Confinement should be designed for each particular organism, based on the ability of the organism to transfer genetic information to other organisms, to disseminate in the environment, to reproduce, and to cause adverse effects.

III-C-1. Confinement Level 1. Level 1 consists of good agricultural practices for the particular organism and site. This includes instructions for all personnel on appropriate record-keeping and monitoring of experiments; it also includes planting, inoculating, reproduction, husbandry,

harvesting, slaughter, and disposal practices that would be used on a well managed farm.

III-C-2. Confinement Level 2. Level 2 consists of the use of good agricultural practices plus increased stringency of the confinement class most appropriate to the organism.

III-C-3. Confinement Level 3. Level 3 consists of the use of good agricultural practices plus increased stringency of two or more of the confinement classes most appropriate to the organism.

III-C-4. Confinement Level 4. Level 4 consists of the use of good agricultural practices plus all available confinement classes appropriate to the organism.

II. Classification of Organisms.

The safety of conducting research outside the laboratory involving biotechnology is relative to the safety of conducting research with the unmodified (wild-type) organism. Genetic modification can increase or decrease the safety either directly by changing the organism, or indirectly from the action of any products or compounds produced by the organism.

Section II-A describes types of genetic modifications included in [relevant to] [discussed in] [appropriate for] these Guidelines that would affect the safety of experiments outside the laboratory. In Section II-B, five (5) statuses are established for grouping unmodified organisms according to their potential for causing adverse effects on human health or on managed or natural ecosystems.

II-A. Genetic modifications.

II-A-1. General. The known methods for genetic modifications of organisms are considered in these Guidelines. Modifications that are expected to result in an organism no different from the unmodified organism with respect to safety, either from a historical or molecular perspective, include the following: those that occur naturally or result from artificial breeding methods, such as, hand pollination, artificial insemination, superovulation or embryo transfer within species, selection of somaclonal variants, or introduction of genetic material consisting solely of same-species DNA or RNA. These are excluded from the scope of the Guidelines. Modifications that,

because of known factors or perceptual uncertainties, are expected to result in organisms that are different from the unmodified organism with respect to safety include but are not limited to: recombinant DNA and genetic manipulations involving transfer of RNA between species accomplished with or without specific molecular gene vectors; physical methods for genetic modification, such as, electroporation, microinjection, and microprojectile procedures; cross-species cell and embryo fusion techniques; and directed mutagenesis.

Genetic modification of an organism, whether included or excluded from the scope of the Guidelines, may result in a change in the status of the organism. The long history of safe conduct with techniques of genetic modification excluded from the scope of the Guidelines indicates that a manageable spectrum of changes in safety can be expected. Techniques for modifying information included within the scope of the Guidelines have no inherently greater or lesser probability for changing the status of the organism than techniques outside the scope of the Guidelines. However, since these techniques enable more precise manipulation of genes and nucleic acids, a closer examination of the molecular nature of the genetic modification and of gene expression is possible. Knowledge of the precise modification may allow better predictability of the change in the modified organism, resulting in a new status. <u>Four</u> types of genetic modification, based on the resulting organism, are presented in this section in order to categorize modifications. <u>This</u>

categorization provides a framework for assessing the likelihood that the modifications will change the status of experimental organisms. ~~The Principal Investigator is initially responsible for assessing the effect of the modification on the status of modified organisms.~~ [Last sentence to be moved to section IV.]

II-A-2. Types of genetic modification.

II-A-2-a. Type 1. Genetic modifications with nucleic acid from any source that result in organisms that are no longer able to express a normal trait condisered hazardous to human health ~~as~~ or to managed or natural ecosystems.

II-A-2-b. Type 2. Genetic modifications that result in organisms that:

II-A-2-b-(1). contain simple deletions or point mutations that are essentially equivalent to those modifications that are likely to occur naturally,

II-A-2-b-(2). contain multiple deletions, complex genomic rearrangements which are unlikely to occur naturally, but do not involve foreign DNA, and

II-A-2-b-(3). contain added nucleic acid solely of the same species, except for sequences known to produce or induce highly hazardous to humans or the environment traits, such as, toxins.

II-A-2-c. Type 3 Genetic modifications that result in organisms containing insertions of nucleic acid from different species. Such organisms may express an intended trait

that has:

II-A-2-c-(1). no phenotypic or genotypic consequence in the field, such as, marker genes.

II-A-2-c-(2). a known phenotypic or genotypic consequence in the field, but that consequence is considered safe, or

II-A-2-c-(3). an unknown phenotypic or genotypic consequence in the field, but any consequence that could be expected is considered safe.

II-A-2-c. Type 4. Genetic modifications with nucleic acid from any source that result in organisms that express an intended trait considered hazardous to human health or to managed or natural ecosystems.

II-B Safety status of unmodified organisms.

II-B-1. Status 1. Organisms generally recognized as compatible with the environment (GRACE). Status 1 organisms have little or no recognized potential for transfer of genetic information to other organisms, or for rapid and widespread dissemination in the environment, or for uncontrollable reproduction, or for (unreasonably) [acute and/or chronic] adverse effects on human health and on managed or natural ecosystems, such as, domesticated organisms and organisms with a long history of use. Examples of Status 1 organisms are cattle, corn, and Rhizobium.

II-B-2. Status 2. Organisms with some potential for transfer of genetic information to other organisms, or for rapid and widespread dissemination in the environment, or for uncontrollable reproduction, or for causing [chronic] adverse effects or (of) [acute adverse effects except those of] predictably low consequence on human health or on managed or natural ecosystems. Examples of Status 2 organisms are laboratory mice, sorghum, and oats.

II-B-3. Status 3. Organisms with potential (a) for (unreasonably) [moderate chronic and/or moderate acute] adverse effects on human health or on managed or natural ecosystems and/or
(b) for transfer of genetic information to other organisms, and/or for rapid and widespread dissemination in the environment, and/or for uncontrollable reproduction. Examples of Status 3 organisms are kudzu, Southern corn leaf blight, and water hyacinth.

II-B-4. Status 4. Organisms with potential (a) for serious (harm to) [chronic and/or acute effects on] human health or on managed or natural ecosystems and (b) for transfer of genetic information to other organisms, or for rapid and widespread dissemination in the environment, or for uncontrollable reproduction. Examples of status 4 organisms are gypsy moth, corn lethal necrosis, and Striga.

II-B-5. Status 5. Organisms with (a) great potential for serious (harm to) [chronic and/or acute effects on] human

health or on managed or natural ecosystems and (b) great potential for transfer of genetic information to other organisms, for rapid and widespread dissemination in the environment, or for uncontrollable reproduction, such as, exotic organisms. Examples of Status 5 organisms are foot and mouth disease virus, plum pox virus and soybean rust fungus.

PROPOSED MATRIX - 1[illegible]/6/89

NEW STATUS ↓ REVIEW + APPROVAL (IV-B) ↓

Exempt Modifications		STATUS 1	STATUS 2	STATUS 3	STATUS 4	STATUS 5
		NA E	NA E	NA E	NA E	NA E
OLD	NEW					
disarm	1	NA —	NS-1 * IBC-N	NS-1 NS-2 * IBC-NA	NS-1 NS-2 NS-3 * NS-4 IBC-A	NS-1 NS-2 NS-3 NS-4 * ABRAC
1	2	NS-1 * IBC-N	NS-1 NS-2 * NS-3 NS-4 IBC-N	NS-1 NS-2 NS-3 * NS-4 ABRAC-3,4 IBC-A/2,1	NS-1 NS-2 NS-3 NS-4 * IBC-A	NA ABRAC
2	3	NS-1 * NS-2 IBC-N	NS-1 NS-2 * NS-3 NS-4 IBC-A	NS-1 NS-2 NS-3 * NS-4 ABRAC ~~IBC-A~~	NS-1 NS-2 NS-3 NS-4 * ABRAC	NA ABRAC
3	4	NS-1 NS-2 * NS-3 IBC-A	NS-2 NS-3 * NS-4 ABRAC	NS-3 NS-4 * NS-5 ABRAC	NS-4 NS-5 * ABRAC	NA ABRAC

* = EXPECTED NEW STATUS FOLLOWING GENETIC MODIFICATION

IV-A

RECOMMENDED CONFINEMENT LEVEL NUMBER TO EQUAL STATUS NO. OR NEW STATUS NO.

E = EXEMPT

IBC-N = IBC NOTIFICATION

IBC-A = IBC PRIOR APPROVAL

ABRAC = REVIEW AND APPROVAL BY ABRAC

January 6, 1989

II. Factors affecting safety of biotechnology research outside the laboratory.

The safety of biotechnological research involving a genetically modified organism and conducted outside the laboratory will be assessed relative to the safety of research which utilizes the unmodified organism. Therefore, types of genetic modification made (Section II-A) and the potential for the unmodified organism to reproduce and do harm to human health or to managed or natural ecosystems (Section II-B) must be considered in establishing appropriate confinement (Section III) for biotechnology research outside the laboratory.

II-A-1. Scope of experimentation covered by the guidelines.

This section of the guidelines covers biotechnology research which results in stable or transmissible genetic changes in organisms when those modified organisms are used in research conducted outside the laboratory Genetic modifications of primary concern and which require guideline compliance are those made through the direct introduction or manipulation of DNA or of RNA, e.g., modification of viral

organisms. Methods for genetic modification or manipulation include, but are not limited to, those involving transfer of recombinant DNA or RNA and accomplished with or without specific molecular vectors and site directed mutagenesis of isolated DNA or RNA which is then reintroduced in an organism.

Genetic modifications which occur through natural reproduction within a species or through random mutation are currently exempt from these guidelines, but principal investigators are nevertheless responsible for the safe conduct of experiments utilizing organisms modified by an exempt method and conducted outside the laboratory, and confinement principles (Section III) appropriate to the organism and the experimental location should be applied to those experiments as well. Furthermore, the IBC should be consulted whenever questions exist regarding safe experimentation outside the laboratory. [Is any justification for exemption required here?]

II-A-2. Types of Genetic Modification

Genetic modifications covered by these guidelines can increase, decrease or have no effect on the level of confinement required for a particular organism when experiments are to be conducted outside the laboratory.

II-A-3-a. Type 1. Genetic modifications that disarm or otherwise decrease the level of confinement needed to safety conduct experiments outside the laboratory utilizing a particular organism.

II-A-3-a-1. A gene or genes specifically known to affect pathogenicity, male or female fertility or competitiveness are deleted or their expression disrupted.

II-A-3-b. Type 2. Genetic modifications expected to have no effect on the level of confinement needed to safely conduct experiments outside the laboratory utilizing a particular organism.

II-A-3-b-1. Simple deletions or point mutations (other than those in II-A-3-a-1).

II-A-3-b-2. Multiple deletions ...

II-A-3-b-3. Insertions of nucleic aid from the same species which produce a gene product not known to result in any hazardous trait with that species.

II-A-3-b-4. Insertions of nucleic acids of no phenotypic or genotypic consequence in the field, e.g., marker genes.

II-A-3-b-5. Insertions of nucleic acid with a known phenotypic or genotypic consequence in the field that is considered safe.

II-A-3-c. Type 3. Genetic modifications which increase level of confinement required for experimentation outside the laboratory with a particular organism.

II-A-3-c-1. Insertion of nucleic acids coding for proteins of unknown function or effect.

II-A-3-c-2. Insertion of nucleic acids coding for proteins known to create hazards to human health or to managed or natural ecosystems.

II-B. Characteristics of organism categories that influence confinement level.

II-B-1. Category 1. Organisms generally recognized as compatible with the environment (GRACE)...(as per existing) These organisms require minimal confinement unless the type of genetic modification increases the need for confinement.

II-B-2. Category 2. Organisms that can transfer genetic information to genetic information to wild species, pests or pathogens or themselves have potential for widespread dissemination in the environment or for uncontrollable reproduction, but consequences of these events are likely to be of low consequence, especially relative to potential benefits that may be anticipated.

II-B-3. Category 3. Organisms that can ...(Same as B-2 to "but"), and consequences of these events are likely to cause

adverse effects on human health or on managed or natural ecosystems which may exceed the potential benefits anticipated.

II-B-4. Category 4. Organisms which are themselves pests or pathogens or which are likely to reproduce, spread or establish in the environment unless high level confinement procedures are implemented, and which pose a clear risk to human health or managed or natural ecosystems which exceeds any likely benefit.

II-B-5. Category 5. Organisms are exotic organisms not indigenous to the area and severe pathogens which should not be used for research outside the laboratory unless or until the risk associated with the organism can be reduced based upon genetic modification or other research that demonstrates the risk to human health and managed or natural ecosystems are acceptable based upon the benefits anticipated from the research.

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